

## Verification of Translation

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hereby declare:

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Dated this 24th day of September, 2008



Reiko Tanaka

[ Document Title ] Patent Application

[ Case number ] PCG-0004

[ Filing Date ] July 9, 2004

[ To ] Commissioner, Japan Patent Office

[ International Patent Classification ] C07K 16/00

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[ Indication of Fee ]		
[ Deposit Account No.]	185396	
[ Amount of Payment ]	¥16,000.-	
[ List of the Documents Submitting ]		
[ Item ]	Patent Claims	1
[ Item ]	Specification	1
[ Item ]	Drawings	1
[ Item ]	Abstract	1
[ General Power of Attorney No. ]	0409940	



## CLAIMS

1. An antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 respectively comprising the amino acid sequence defined by the sequences of any one of (1) - (12):

- (1) SEQ ID NO: 123, 124 and 125 (GC33);
- (2) SEQ ID NO: 109, 110 and 111 (M11F1);
- (3) SEQ ID NO: 106, 107 and 108 (M3B8);
- (4) SEQ ID NO: 132, 133 and 134 (GC199);
- (5) SEQ ID NO: 106, 135 and 136 (GC202);
- (6) SEQ ID NO: 126, 127 and 128 (GC179);
- (7) SEQ ID NO: 129, 130 and 131 (GC194);
- (8) SEQ ID NO: 103, 104 and 105 (M13B3);
- (9) SEQ ID NO: 118, 121 and 122 (L9G11);
- (10) SEQ ID NO: 115, 116 and 117 (M6B1);
- (11) SEQ ID NO: 112, 113 and 114 (M5B9);
- (12) SEQ ID NO: 118, 119 and 120 (M10D2).

2. An antibody comprising a light chain variable region having CDRs 1, 2 and 3 respectively comprising the amino acid sequence defined by the sequences of any one of (1) - (13):

- (1) SEQ ID NO: 143, 144 and 158 (GC33);
- (2) SEQ ID NO: 143, 144 and 145 (M11F1);
- (3) SEQ ID NO: 140, 141 and 142 (M3B8);
- (4) SEQ ID NO: 167, 168 and 169 (GC199);

- (5) SEQ ID NO: 170, 144 and 171 (GC202);
- (6) SEQ ID NO: 159, 160 and 161 (GC179);
- (7) SEQ ID NO: 162, 147 and 163 (GC194 (1));
- (8) SEQ ID NO: 164, 165 and 166 (GC194 (2));
- (9) SEQ ID NO: 137, 138 and 139 (M13B3);
- (10) SEQ ID NO: 155, 156 and 157 (L9G11);
- (11) SEQ ID NO: 149, 150 and 151 (M6B1);
- (12) SEQ ID NO: 146, 147 and 148 (M5B9);
- (13) SEQ ID NO: 152, 153 and 154 (M10D2).

3. An antibody selected from the group consisting of any one of (1) - (13):

(1) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 143, 144 and 158, respectively;

(2) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 109, 110 and 111, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 143, 144 and 145, respectively;

(3) an antibody comprising a heavy chain variable region

having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 106, 107 and 108, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 140, 141 and 142, respectively;

(4) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 132, 133 and 134, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 167, 168 and 169, respectively;

(5) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 106, 135 and 136, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 170, 144 and 171, respectively;

(6) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 126, 127 and 128, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 159, 160 and 161, respectively;

(7) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set

forth in SEQ ID NO: 129, 130 and 131, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 162, 147 and 163, respectively;

(8) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 129, 130 and 131, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 164, 165 and 166, respectively;

(9) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 103, 104 and 105, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 137, 138 and 139, respectively;

(10) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 118, 121 and 122, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 155, 156 and 157, respectively;

(11) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 115, 116 and 117, respectively, and a light

chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 149, 150 and 151, respectively;

(12) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 112, 113 and 114, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 146, 147 and 148, respectively; and

(13) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 118, 119 and 120, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 152, 153 and 154, respectively.

4. An antibody having a heavy chain variable region of any one of (1) - (7):

(1) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 84;

(2) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 85;

(3) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 86;

(4) a heavy chain variable region comprising the amino acid

sequence set forth in SEQ ID NO: 87;

(5) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 88;

(6) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 89; or

(7) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 90.

5. An antibody having a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92.

6. An antibody selected from the group consisting of the antibody of any one of (1) - (7):

(1) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 84 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92;

(2) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 85 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92;

(3) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 86 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92;

(4) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 87 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92;

(5) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 88 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92;

(6) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 89 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92; and

(7) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 90 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92.

7. An antibody having an activity equivalent to the activity of the antibody of any one of Claims 1-6, wherein one or more amino acid residues are substituted, deleted or added and/or inserted from the amino acid sequences set forth in any one of Claims 1-6.

8. The antibody as claimed in any one of Claims 1-7 which is a humanized antibody.

9. A humanized antibody capable of binding to glypican 3.
10. An antibody capable of binding to a peptide consisting of the sequence of the amino acid residues 524 - 563 of glypican 3.
11. An antibody capable of binding to a peptide consisting of the sequence of the amino acid residues 537 - 563 of glypican 3.
12. The antibody as claimed in Claim 10 or 11 which does not bind to a peptide consisting of the sequence of the amino acid residues 550 - 563 of glypican 3.
13. An antibody capable of binding to a peptide consisting of the sequence of the amino acid residues 544 - 553 of glypican 3.
14. An antibody capable of binding to a peptide consisting of the sequence of the amino acid residues 546 - 551 of glypican 3.
15. The antibody as claimed in any one of Claims 9-14 which is a humanized antibody.



16. An antibody capable of binding to an epitope to which a second antibody is capable of binding, wherein said second antibody comprises a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 143, 144 and 158, respectively.

17. An antibody capable of binding to glypican 3 which has a high CDC activity against a cell expressing glypican 3.

18. An antibody capable of binding to glypican 3 which has a high ADCC activity against a cell expressing glypican 3.

19. Apolynucleotide coding for a heavy chain variable region or a light chain variable region of the antibody as claimed in any one of Claims 1-18.

20. The polynucleotide as claimed in Claim 18 having the sequence set forth in SEQ ID NOS: 11-21, 33-43, 55-59, 65-70 and 77-83.

21. A cell-growth inhibitor comprising as an active

ingredient the antibody as claimed in any one of Claims 1-18.

22. An anticancer agent comprising as an active ingredient the antibody as claimed in any one of Claims 1-18.

23. The anticancer agent as claimed in Claim 22 for treatment of hepatoma.

24. A peptide comprising the amino acid sequence of the amino acid residues 524 - 563 of glypican 3.

25. A peptide comprising the amino acid sequence of the amino acid residues 537 - 563 of glypican 3.

26. A peptide comprising the amino acid sequence of the amino acid residues 544 - 553 of glypican 3.

27. A peptide comprising the amino acid sequence of the amino acid residues 546 - 551 of glypican 3.

28. An antibody comprising a light chain variable region having CDRs 1, 2 and 3 respectively comprising the amino acid sequence defined by the sequence of (1) - (15) below:

(1) SEQ ID NO: 174, 144 and 158

(2) SEQ ID NO: 175, 144 and 158

- (3) SEQ ID NO: 176, 144 and 158
- (4) SEQ ID NO: 177, 144 and 158
- (5) SEQ ID NO: 178, 144 and 158
- (6) SEQ ID NO: 179, 144 and 158
- (7) SEQ ID NO: 180, 144 and 158
- (8) SEQ ID NO: 181, 144 and 158
- (9) SEQ ID NO: 182, 144 and 158
- (10) SEQ ID NO: 183, 144 and 158
- (11) SEQ ID NO: 184, 144 and 158
- (12) SEQ ID NO: 185, 144 and 158
- (13) SEQ ID NO: 186, 144 and 158
- (14) SEQ ID NO: 187, 144 and 158
- (15) SEQ ID NO: 188, 144 and 158.

29. An antibody selected from the group consisting of the antibody of (1) - (15):

(1) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 174, 144 and 158, respectively;

(2) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light

chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 175, 144 and 158, respectively;

(3) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 176, 144 and 158, respectively;

(4) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 177, 144 and 158, respectively;

(5) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 178, 144 and 158, respectively;

(6) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the

amino acid sequence set forth in SEQ ID NO: 179, 144 and 158, respectively;

(7) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 180, 144 and 158, respectively;

(8) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 181, 144 and 158, respectively;

(9) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 182, 144 and 158, respectively;

(10) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 183, 144 and 158,

respectively;

(11) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 184, 144 and 158, respectively;

(12) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124, and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 185, 144, and 158, respectively;

(13) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124, and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 186, 144 and 158, respectively;

(14) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 187, 144 and 158, respectively; and

(15) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 188, 144 and 158, respectively.

30. An antibody having a light chain variable region selected from (1) - (15):

(1) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 191;

(2) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 192;

(3) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 193;

(4) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 194;

(5) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 195;

(6) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 196;

(7) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 197;

(8) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 198;

- (9) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 199;
- (10) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 200;
- (11) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 201;
- (12) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 202;
- (13) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 203;
- (14) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 204; and
- (15) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 205.

31. An antibody having a light chain variable region selected from the group consisting of (1) - (15):

- (1) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 191;
- (2) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 192;
- (3) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 193;
- (4) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 194;



(5) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 195;

(6) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 196;

(7) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 197;

(8) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 198;

(9) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 199;

(10) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 200;

(11) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 201;

(12) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 202;

(13) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 203;

(14) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 204; and

(15) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 205;

and a heavy chain variable region selected from the group consisting of (1) - (7):

(1) a heavy chain variable region comprising the amino acid

sequence set forth in SEQ ID NO: 84;

(2) a heavy chain variable region comprising; the amino acid sequence set forth in SEQ ID NO: 85;

(3) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 86;

(4) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 87;

(5) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 88;

(6) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 89; and

(7) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 90.

32. The antibody as claimed in any one of Claims 28 - 31 which is a human antibody.

#### TITLE OF THE INVENTION

ANTI-GLYPICAN 3 ANTIBODY

#### BACKGROUND OF THE INVENTION

##### Field of the Invention

The present invention relates to an anti-glypican 3 antibody, a cell growth inhibitor and an anticancer agent containing the antibody as an active ingredient.

#### Description of Related Art

Glypican 3 (GPC3) is one of the glypican family of heparan sulfate proteoglycans that are present on cell surfaces. It is suggested that GPC3 may be involved in cell division in development or cancer cell growth, however, its function has not been well elucidated yet.

It has been found that a certain type of antibody binding to GPC3 has a cell growth-inhibiting activity via an antibody-dependent cell-mediated cytotoxicity (ADCC) activity and a complement-dependent cytotoxicity (CDC) activity (International Patent Application WO 2003/000883). In addition, it has been suggested that GPC3 is cleaved in vivo and secreted into blood as a secreted form of GPC3, and the diagnosis of cancers may be possible by using an antibody capable of detecting the secreted form of GPC3 (International Patent Applications WO 2004/022739, WO 03/100429 and WO 2004/018667).

When developing an anticancer agent based on the cytotoxicity activity of an antibody, it is preferred that the antibody to be used has high ADCC activity or CDC activity. Accordingly, an anti-GPC3 antibody having a high cytotoxicity activity has been desired as an antibody recognizing GPC3.

List of related documents:

1. WO2003/000883
2. WO 2004/022739
3. WO 03/100429
4. WO 2004/018667).

An object of the present invention is to provide an anti-GPC3 antibody having a higher ADCC activity and CDC activity compared with those of a conventional antibody.

#### SUMMARY OF THE INVENTION

The present inventors have succeeded in obtaining an antibody having a higher cytotoxicity activity compared with that of a conventional anti-glypican 3 antibody. Furthermore, they analyzed epitopes for such an antibody and succeeded in determining the regions on GPC 3 recognized by the antibody with a high cytotoxicity activity.

In one aspect, the present invention provides an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 of any one of (1) - (12):

1. An antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 respectively comprising the amino acid sequence defined by the sequences of any one of (1) - (12):

(1) SEQ ID NO: 123, 124 and 125 (GC33);

(2) SEQ ID NO: 109, 110 and 111 (M11F1);

- (3) SEQ ID NO: 106, 107 and 108 (M3B8);
- (4) SEQ ID NO: 132, 133 and 134 (GC199);
- (5) SEQ ID NO: 106, 135 and 136 (GC202);
- (6) SEQ ID NO: 126, 127 and 128 (GC179);
- (7) SEQ ID NO: 129, 130 and 131 (GC194);
- (8) SEQ ID NO: 103, 104 and 105 (M13B3);
- (9) SEQ ID NO: 118, 121 and 122 (L9G11);
- (10) SEQ ID NO: 115, 116 and 117 (M6B1);
- (11) SEQ ID NO: 112, 113 and 114 (M5B9);
- (12) SEQ ID NO: 118, 119 and 120 (M10D2).

In another aspect, the invention provides an antibody comprising a light chain variable region having CDRs 1, 2 and 3 of any one of (1) - (13):

- (1) SEQ ID NO: 143, 144 and 158 (GC33);
- (2) SEQ ID NO: 143, 144 and 145 (M11F1);
- (3) SEQ ID NO: 140, 141 and 142 (M3B8);
- (4) SEQ ID NO: 167, 168 and 169 (GC199);
- (5) SEQ ID NO: 170, 144 and 171 (GC202);
- (6) SEQ ID NO: 159, 160 and 161 (GC179);
- (7) SEQ ID NO: 162, 147 and 163 (GC194 (1));
- (8) SEQ ID NO: 164, 165 and 166 (GC194 (2));
- (9) SEQ ID NO: 137, 138 and 139 (M13B3);
- (10) SEQ ID NO: 155, 156 and 157 (L9G11);
- (11) SEQ ID NO: 149, 150 and 151 (M6B1);
- (12) SEQ ID NO: 146, 147 and 148 (M5B9);

(13) SEQ ID NO: 152, 153 and 154 (M10D2).

Preferably, the antibody of the invention is selected from the group consisting of any one of (1) - (13):

(1) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 143, 144 and 158, respectively (GC33);

(2) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 109, 110 and 111, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 143, 144 and 145, respectively (M11F1);

(3) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 106, 107 and 108, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 140, 141 and 142, respectively (M3B8);

(4) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 132, 133 and 134, respectively, and a light

chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 167, 168 and 169, respectively (GC199);

(5) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 106, 135 and 136, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 170, 144 and 171, respectively (GC202);

(6) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 126, 127 and 128, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 159, 160 and 161, respectively (GC179);

(7) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 129, 130 and 131, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 162, 147 and 163, respectively (GC194(1));

(8) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 129, 130 and 131, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the

amino acid sequence set forth in SEQ ID NO: 164, 165 and 166, respectively (GC194(2));

(9) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 103, 104 and 105, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 137, 138 and 139, respectively (M13B3);

(10) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 118, 121 and 122, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 155, 156 and 157, respectively (L9G11);

(11) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 115, 116 and 117, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 149, 150 and 151, respectively (M6B1);

(12) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 112, 113 and 114, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 146, 147 and 148,



respectively (M5B9); and

(13) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 118, 119 and 120, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 152, 153 and 154, respectively (M10D2).

In another aspect, the invention provides an antibody having a heavy chain variable region of any one of (1) - (7):

(1) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 84 (GC33 VHver.a);

(2) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 85 (GC33 VHver.c);

(3) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 86 (GC33 VHver.f);

(4) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 87 (GC33 VHver.h);

(5) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 88 (GC33 VHver.i);

(6) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 89 (GC33 VHver.j); or

(7) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 90 (GC33 VHver.k).

In another aspect, the invention provides an antibody having a light chain variable region comprising the amino acid

sequence set forth in SEQ ID NO: 92.

Preferably, the antibody of the invention is selected from the group consisting of the antibody of any one of (1) - (7):

(1) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 84 (GC33 VHver.a) and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VLver.a);

(2) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 85 (GC33 VHver.c) and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VLver.a);

(3) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 86 (GC33 VHver.f) and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VLver.a);

(4) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 87 (GC33 VHver.h) and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VLver.a);

(5) an antibody comprising a heavy chain variable region

comprising the amino acid sequence set forth in SEQ ID NO: 88 (GC33 VHver.i) and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VLver.a);

(6) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 89 (GC33 VHver.j) and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VLver.a); and

(7) an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 90 (GC33 VHver.k) and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VLver.a).

In another aspect, the invention provides an antibody comprising a light chain variable region having CDRs 1, 2 and 3 of any one of (1) - (15):

- (1) SEQ ID NO: 174, 144 and 158
- (2) SEQ ID NO: 175, 144 and 158
- (3) SEQ ID NO: 176, 144 and 158
- (4) SEQ ID NO: 177, 144 and 158
- (5) SEQ ID NO: 178, 144 and 158
- (6) SEQ ID NO: 179, 144 and 158
- (7) SEQ ID NO: 180, 144 and 158
- (8) SEQ ID NO: 181, 144 and 158

- (9) SEQ ID NO: 182, 144 and 158
- (10) SEQ ID NO: 183, 144 and 158
- (11) SEQ ID NO: 184, 144 and 158
- (12) SEQ ID NO: 185, 144 and 158
- (13) SEQ ID NO: 186, 144 and 158
- (14) SEQ ID NO: 187, 144 and 158
- (15) SEQ ID NO: 188, 144 and 158.

In another aspect, the invention provides an antibody selected from the group consisting of the antibody of (1) - (15):

(1) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 174, 144 and 158, respectively;

(2) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 175, 144 and 158, respectively;

(3) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light

chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 176, 144 and 158, respectively;

(4) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 177, 144 and 158, respectively;

(5) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 178, 144 and 158, respectively;

(6) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 179, 144 and 158, respectively;

(7) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the

amino acid sequence set forth in SEQ ID NO: 180, 144 and 158, respectively;

(8) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 181, 144 and 158, respectively;

(9) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 182, 144 and 158, respectively;

(10) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 183, 144 and 158, respectively;

(11) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 184, 144 and 158,

respectively;

(12) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124, and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 185, 144, and 158, respectively;

(13) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124, and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 186, 144 and 158, respectively;

(14) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 187, 144 and 158, respectively; and

(15) an antibody comprising a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 188, 144 and 158, respectively.

In further aspect, the invention provides an antibody having a light chain variable region selected from (1) - (15):

(1) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 191;

(2) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 192;

(3) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 193;

(4) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 194;

(5) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 195;

(6) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 196;

(7) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 197;

(8) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 198;

(9) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 199;

(10) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 200;

(11) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 201;

(12) a light chain variable region comprising the amino acid



sequence set forth in SEQ ID NO: 202;

(13) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 203;

(14) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 204; and

(15) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 205.

In another aspect, the invention provides an antibody having a light chain variable region selected from the group consisting of (1) - (15):

(1) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 191;

(2) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 192;

(3) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 193;

(4) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 194;

(5) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 195;

(6) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 196;

(7) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 197;

(8) a light chain variable region comprising the amino acid

sequence set forth in SEQ ID NO: 198;

(9) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 199;

(10) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 200;

(11) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 201;

(12) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 202;

(13) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 203;

(14) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 204; and

(15) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 205;

and a heavy chain variable region selected from the group consisting of (1) - (7):

(1) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 84;

(2) a heavy chain variable region comprising; the amino acid sequence set forth in SEQ ID NO: 85;

(3) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 86;

(4) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 87;

- (5) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 88;
- (6) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 89; and
- (7) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 90.

Also the invention features an antibody having an activity equivalent to the activity of the antibody described above, wherein one or more amino acid residues are substituted, deleted or added and/or inserted from the amino acid sequences described above. Preferably, the antibody of the invention is a humanized antibody. Thus, in another aspect, the invention provides a humanized antibody capable of binding to glypican 3.

In further aspect, the invention provides an antibody capable of binding to a peptide consisting of the sequence of the amino acid residues 524 - 563 of glypican 3. Preferably, the antibody of the invention is capable of binding to a peptide consisting of the sequence of the amino acid residues 537 - 563 of glypican 3. More preferably, the antibody of the invention does not bind to a peptide consisting of the sequence of the amino acid residues 550 - 563 of glypican 3. Preferably, the antibody is capable of binding to a peptide consisting of the sequence of the amino acid residues 544 - 553 of glypican 3 or a peptide consisting of the sequence of the amino acid

residues 546 - 551 of glypican 3.

In still another aspect, the invention provides an antibody capable of binding to an epitope to which a second antibody is capable of binding, wherein said second antibody comprises a heavy chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 123, 124 and 125, respectively, and a light chain variable region having CDRs 1, 2 and 3 comprising the amino acid sequence set forth in SEQ ID NO: 143, 144 and 158, respectively. Namely, the antibody of the invention is capable of competing in binding to GPC3 with the second antibody.

In a preferred embodiment, the antibody of the invention is capable of binding to glypican 3 and has a high CDC activity against a cell expressing glypican 3 and/or has a high ADCC activity against a cell expressing glypican 3.

In another aspect, the invention provides a polynucleotide coding for a heavy chain variable region or a light chain variable region of the antibody of the invention.

Preferably, the polynucleotide of the invention has the sequence set forth in SEQ ID NOs: 11-21, 33-43, 55-59, 65-70 and 77-83.

In further aspect, the invention provides a peptide comprising the sequence of the amino acid residues 524 - 563 of glypican 3, the sequence of the amino acid residues 537 - 563 of glypican 3, the sequence of the amino acid residues 544

- 553 of glypican 3 or the amino acid sequence of the amino acid residues 546 - 551 of glypican 3.

In still another aspect, the invention provides a cell-growth inhibitor and an anticancer agent comprising as an active ingredient the antibody of the invention. Preferably, the anticancer agent of the invention is used for treatment of hepatoma.

#### DETAILED DESCRIPTION OF THE INVENTION

##### Antibody

The present invention provides antibodies described in the following (I) to (XI).

(I) An antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: in any of the following (1) to (12):

- (1) SEQ ID NOs: 123, 124 and 125 (GC33),
- (2) SEQ ID NOs: 109, 110 and 111 (M11F1),
- (3) SEQ ID NOs: 106, 107 and 108 (M3B8),
- (4) SEQ ID NOs: 132, 133 and 134 (GC199),
- (5) SEQ ID NOs: 106, 135 and 136 (GC202),
- (6) SEQ ID NOs: 126, 127 and 128 (GC179),
- (7) SEQ ID NOs: 129, 130 and 131 (GC194),
- (8) SEQ ID NOs: 103, 104 and 105 (M13B3),
- (9) SEQ ID NOs: 118, 121 and 122 (L9G11),
- (10) SEQ ID NOs: 115, 116 and 117 (M6B1),

(11) SEQ ID NOs: 112, 113 and 114 (M5B9), and

(12) SEQ ID NOs: 118, 119 and 120 (M10D2).

Among the antibodies described in (1) to (12), preferred are the antibodies described in (1) to (8), more preferred are the antibodies described in (1) to (5), and particularly preferred is the antibody described in (1). The antibodies described in (1) to (8) recognize the C-terminal peptide of glypican 3 (a peptide comprising the 374th amino acid to the 580th amino acid of glypican 3); and are useful as a therapeutic antibody. In addition, the antibodies described in (9) to (12) recognize the N-terminal peptide of glypican 3 (a peptide comprising from the 1st amino acid to the 373rd amino acid of glypican 3); and are useful as a diagnostic antibody.

(II) An antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: in any of the following (1) to (13):

(1) SEQ ID NOs: 143, 144 and 158 (GC33),

(2) SEQ ID NOs: 143, 144 and 145 (M11F1),

(3) SEQ ID NOs: 140, 141 and 142 (M3B8),

(4) SEQ ID NOs: 167, 168 and 169 (GC199),

(5) SEQ ID NOs: 170, 144 and 171 (GC202),

(6) SEQ ID NOs: 159, 160 and 161 (GC179),

(7) SEQ ID NOs: 162, 147 and 163 (GC194 (1)),

(8) SEQ ID NOs: 164, 165 and 166 (GC194 (2)),

(9) SEQ ID NOs: 137, 138 and 139 (M13B3),

- (10) SEQ ID NOs: 155, 156 and 157 (L9G11),
- (11) SEQ ID NOs: 149, 150 and 151 (M6B1),
- (12) SEQ ID NOs: 146, 147 and 148 (M5B9), and
- (13) SEQ ID NOs: 152, 153 and 154 (M10D2).

Among the antibodies described in (1) to (13), preferred are the antibodies described in (1) to (8), more preferred are the antibodies described in (1) to (5), and particularly preferred is the antibody described in (1). The antibodies described in (1) to (8) recognize the C-terminal peptide of glypican 3 (a peptide comprising from the 374th amino acid to the 580th amino acid of glypican 3); and are useful as a therapeutic antibody. In addition, the antibodies described in (9) to (13) recognize the N-terminal peptide of glypican 3 (a peptide comprising from the 1st amino acid to the 373rd amino acid of glypican 3); and are useful as a diagnostic antibody.

(III) An antibody selected from the group consisting of the antibodies described in the following (1) to (13):

- (1) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 143, 144 and 158 (GC33),
- (2) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth

in SEQ ID NOs: 109, 110 and 111, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 143, 144 and 145 (M11F1),

(3) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 106, 107 and 108, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 140, 141 and 142 (M3B8),

(4) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 132, 133 and 134, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 167, 168 and 169 (GC199),

(5) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 106, 135 and 136, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 170, 144 and 171 (GC202),

(6) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 126, 127 and 128, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 159, 160 and 161 (GC179),

(7) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth



in SEQ ID NOs: 129, 130 and 131, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 162, 147 and 163 (GC194 (1)),

(8) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 129, 130 and 131, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 164, 165 and 166 (GC194 (2)),

(9) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 103, 104 and 105, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 137, 138 and 139 (M13B3),

(10) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 118, 121 and 122, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 155, 156 and 157 (L9G11),

(11) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 115, 116 and 117, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 149, 150 and 151 (M6B1),

(12) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth

in SEQ ID NOs: 112, 113 and 114, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 146, 147 and 148 (M5B9),  
(13) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 118, 119 and 120, and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 152, 153 and 154 (M10D2).

Among the antibodies described in (1) to (13), preferred are the antibodies described in (1) to (8), more preferred are the antibodies described in (1) to (5), and particularly preferred is the antibody described in (1). The antibodies described in (1) to (8) recognize the C-terminal peptide of glypican 3 (a peptide comprising from the 374th amino acid to the 580th amino acid of glypican 3); and are useful as a therapeutic antibody. In addition, the antibodies described in (9) to (13) recognize the N-terminal peptide of glypican 3 (a peptide comprising from the 1st amino acid to the 373rd amino acid of glypican 3); and are useful as a diagnostic antibody.

(IV) An antibody having a heavy chain variable region described in any of the following (1) to (7):

(1) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 84 (GC33 VH ver.a),

(2) a heavy chain variable region containing the amino acid

sequence set forth in SEQ ID NO: 85 (GC33 VH ver.c),  
(3) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 86 (GC33 VH ver.f),  
(4) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 87 (GC33 VH ver.h),  
(5) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 88 (GC33 VH ver.i),  
(6) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 89 (GC33 VH ver.j), and  
(7) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 90 (GC33 VH ver.k).

Among the antibodies described in (1) to (7), particularly preferred are the antibodies described in (2) to (7).

(V) An antibody having a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VL ver.a).

(VI) An antibody selected from the group consisting of the antibodies described in the following (1) to (7):

(1) an antibody having a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 84 (GC33 VH ver.a) and a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VL ver.a),  
(2) an antibody having a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 85 (GC33 VH

ver.c) and a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VL ver.a),

(3) an antibody having a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 86 (GC33 VH ver.f) and a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VL ver.a),

(4) an antibody having a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 87 (GC33 VH ver.h) and a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VL ver.a),

(5) an antibody having a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 88 (GC33 VH ver.i) and a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VL ver.a),

(6) an antibody having a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 89 (GC33 VH ver.j) and a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VL ver.a), and

(7) an antibody having a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 90 (GC33 VH ver.k) and a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 92 (GC33 VL ver.a).

Among the antibodies described in (1) to (7), particularly preferred are the antibodies described in (2) to (7).

(VII) An antibody described in any of the following (1) to (15) :

(1) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 174, 144 and 158,

(2) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 175, 144 and 158,

(3) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 176, 144 and 158,

(4) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 177, 144 and 158,

(5) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 178, 144 and 158,

(6) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 179, 144 and 158,

(7) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 180, 144 and 158,

(8) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 181, 144 and 158,

(9) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 182, 144 and 158,

(10) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 183, 144 and 158,

(11) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 184, 144 and 158,

(12) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 185, 144 and 158,

(13) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 186, 144 and 158,

(14) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 187, 144 and 158, and

(15) an antibody containing light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 188, 144 and 158.

(VIII) An antibody described in any of the following (1) to (15):

(1) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth

in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 174, 144 and 158,

(2) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 175, 144 and 158,

(3) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 176, 144 and 158,

(4) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 177, 144 and 158,

(5) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 178, 144 and 158,

(6) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth

in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 179, 144 and 158,

(7) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 180, 144 and 158,

(8) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 181, 144 and 158,

(9) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 182, 144 and 158,

(10) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 183, 144 and 158,

(11) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth



in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 184, 144 and 158,

(12) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 185, 144 and 158,

(13) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 186, 144 and 158,

(14) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 187, 144 and 158, and

(15) an antibody containing heavy chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 123, 124 and 125 and light chain variable regions having CDRs 1, 2 and 3 consisting of the amino acid sequences set forth in SEQ ID NOs: 188, 144 and 158.

(IX) An antibody described in any of the following (1) to (15):

(1) an antibody having a light chain variable region containing

the amino acid sequence set forth in SEQ ID NO: 191,  
(2) an antibody having a light chain variable region containing  
the amino acid sequence set forth in SEQ ID NO: 192,  
(3) an antibody having a light chain variable region containing  
the amino acid sequence set forth in SEQ ID NO: 193,  
(4) an antibody having a light chain variable region containing  
the amino acid sequence set forth in SEQ ID NO: 194,  
(5) an antibody having a light chain variable region containing  
the amino acid sequence set forth in SEQ ID NO: 195,  
(6) an antibody having a light chain variable region containing  
the amino acid sequence set forth in SEQ ID NO: 196,  
(7) an antibody having a light chain variable region containing  
the amino acid sequence set forth in SEQ ID NO: 197,  
(8) an antibody having a light chain variable region containing  
the amino acid sequence set forth in SEQ ID NO: 198,  
(9) an antibody having a light chain variable region containing  
the amino acid sequence set forth in SEQ ID NO: 199,  
(10) an antibody having a light chain variable region  
containing the amino acid sequence set forth in SEQ ID NO: 200,  
(11) an antibody having a light chain variable region  
containing the amino acid sequence set forth in SEQ ID NO: 201,  
(12) an antibody having a light chain variable region  
containing the amino acid sequence set forth in SEQ ID NO: 202,  
(13) an antibody having a light chain variable region  
containing the amino acid sequence set forth in SEQ ID NO: 203,

(14) an antibody having a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 204, and

(15) an antibody having a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 205.

(X) An antibody having a light chain variable region selected from the group consisting of the light chain variable regions described in the following (1) to (15):

(1) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 191,

(2) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 192,

(3) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 193,

(4) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 194,

(5) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 195,

(6) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 196,

(7) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 197,

(8) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 198,

(9) a light chain variable region containing the amino acid

sequence set forth in SEQ ID NO: 199,

(10) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 200,

(11) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 201,

(12) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 202,

(13) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 203,

(14) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 204, and

(15) a light chain variable region containing the amino acid sequence set forth in SEQ ID NO: 205, and a heavy chain variable region selected from the group consisting of the heavy chain variable regions described in the following (1) to (7):

(1) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 84,

(2) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 85,

(3) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 86,

(4) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 87,

(5) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 88,

(6) a heavy chain variable region containing the amino acid sequence set forth in SEQ ID NO: 89, and

(XI) An antibody, in which one or more amino acids have been replaced, deleted, added and/or inserted in the amino acid sequence described in any one of the above-mentioned (I) to (X), and which has an activity equivalent to that of the antibody described in any of (I) to (X).

In the present invention, the activity equivalent to that of the antibody described in any of (I) to (X) means that the binding activity to a human glypican 3 antibody or the cytotoxicity activity on a cell that expresses human glypican 3 (e.g., HepG2 or a recombinant CHO cells expressing human glypican 3, etc.) is equivalent.

#### Humanized antibody

One preferred embodiment of the antibody according to the present invention is a humanized antibody that binds to glypican 3. The humanized antibody can be prepared by using a known method.

The humanized antibody is also referred to as a reshaped human antibody, which is made by transplanting the complementarity determining region (CDR) of an antibody of a non-human mammal, for example a mouse antibody, into the CDR of a human antibody. The general recombinant DNA technology for preparation of such antibodies is also known (see European

Patent Application EP 125023 and International Patent Application WO 96/02576).

Specifically, for example, in the case where a CDR is derived from a mouse antibody, a DNA sequence which has been designed to link the CDRs of the mouse antibody with the framework region (FR) of a human antibody is synthesized by the PCR method using several oligonucleotides as primers, which have been prepared so as to have portions overlapping with one another at both ends of the CDR and the FR (see the method described in International Patent Application WO 98/13388).

As for the framework region of a human antibody to be linked with the CDR, the one which allows a complementarity determining region to form a favorable antigen-binding site is selected. If necessary, an amino acid in the framework region of a variable region of the antibody may be replaced so that the complementarity determining region of a reshaped human antibody may form an appropriate antigen-binding site (Sato, K. et al., Cancer Res. (1993) 53, 851-856).

The C region of a human antibody may be used as the C region of a chimeric antibody or a humanized antibody, for example, C $\gamma$ 1, C $\gamma$ 2, C $\gamma$ 3, and C $\gamma$ 4 may be used in the H chain, and C $\kappa$  and C $\lambda$  may be used in the L chain. The C region of a human antibody may also be modified in order to improve the stability of the antibody or the production thereof. The human antibody to be used in the humanization may be any isotype of human

antibody, for example, IgG, IgM, IgA, IgE and IgD, preferably, IgG, more preferably IgG1 or IgG3, and particularly preferably IgG1. the present invention IgG1 is effective when an antibody is used as an anticancer agent in terms of having a high cytotoxicity activity (Chemical immunology, 65: 88 (1997)).

In addition, after the humanized antibody is prepared, an amino acid in a variable region (e.g., FR) or a constant region may be replaced with another amino acid.

The origin of the CDR in a humanized antibody is not particularly limited, and the CDR may be derived from any animals. For example, it is possible to use a sequence derived from a mouse antibody, a rat antibody, a rabbit antibody, a camel antibody or the like. Preferred is a CDR sequence of a mouse antibody.

With regard to the humanization of an antibody, it is generally difficult to humanize it while maintaining the agonist activity of the original antibody. In the present invention, however, a humanized antibody having an agonist activity equivalent to that of the original mouse antibody was successfully acquired. Since the antigenicity of the humanized antibody in the human body is reduced, it is useful in administering it into the human for a therapeutic purpose.

Preferred examples of the humanized anti- glypican 3 antibody in the present invention include, for example, an antibody having a heavy chain variable region set forth in SEQ

ID NO: 84 (GC33 VH ver.a), SEQ ID NO: 85 (GC33 VH ver.c), SEQ ID NO: 86 (GC33 VH ver.f), SEQ ID NO: 87 (GC33 VH ver.h), SEQ ID NO: 88 (GC33 VH ver.i), SEQ ID NO: 89 (GC33 VH ver.j) or SEQ ID NO: 90 (GC33 VH ver.k) or an antibody having a light chain variable region set forth in SEQ ID NO: 92 (GC33 VL ver.a). Particularly preferred examples thereof include an antibody having a heavy chain variable region set forth in SEQ ID NO: 84 (GC33 VH ver.a), SEQ ID NO: 85 (GC33 VH ver.c), SEQ ID NO: 86 (GC33 VH ver.f), SEQ ID NO: 87 (GC33 VH ver.h), SEQ ID NO: 88 (GC33 VH ver.i), SEQ ID NO: 89 (GC33 VH ver.j) or SEQ ID NO: 90 (GC33 VH ver.k) and a light chain variable region set forth in SEQ ID NO: 92 (GC33 VL ver.a).

A preferred embodiment of the antibody according to the present invention is an antibody that binds to the epitope to which the antibody set forth in any of the following (1) to (8) binds:

(1) an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 62 and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 73 (GC33),

(2) an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 26 and a light chain variable region the amino acid sequence set forth in SEQ ID NO: 48 (M11F1),

(3) an antibody containing a heavy chain variable region having



the amino acid sequence set forth in SEQ ID NO: 25 and a light chain variable region the amino acid sequence set forth in SEQ ID NO: 47 (M3B8),

(4) an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 60 and a light chain variable region the amino acid sequence set forth in SEQ ID NO: 71 (GC199),

(5) an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 61 and a light chain variable region the amino acid sequence set forth in SEQ ID NO: 72 (GC202),

(6) an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 63 and a light chain variable region the amino acid sequence set forth in SEQ ID NO: 74 (GC179),

(7) an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 64 and a light chain variable region the amino acid sequence set forth in SEQ ID NO: 75 (GC194 (1)), and

(8) an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 64 and a light chain variable region the amino acid sequence set forth in SEQ ID NO: 76 (GC194 (2)). More preferred is an antibody that binds to the epitope to which the antibody described in any of (1) to (5) binds, and particularly preferred is an antibody that

binds to the epitope to which the antibody described in (1) binds.

The antibody that binds to the epitope to which any of the above-mentioned antibodies bind is useful because it has a particularly high cytotoxicity.

The antibody described in any of (1) to (7) binds to a region from the 524th amino acid to the 580th amino acid of human glypican 3. In particular, it binds to a region from the 524th amino acid to the 563rd amino acid. The antibody described in any of (1) to (5) binds to a region from the 537th amino acid to the 563rd amino acid of human glypican 3. The antibody described in (1) binds to a region from the 544th amino acid to the 553rd amino acid of human glypican 3. In particular, it binds to a region from the 546th amino acid to the 551st amino acid.

The antibodies recognizing the above-mentioned epitopes have a high cytotoxicity, therefore they are useful in the treatment of a disease such as cancer. In particular, the antibody which binds to a region from the 546th amino acid to the 551st amino acid is useful as it has a particularly high cytotoxicity

Accordingly, the present invention includes the antibodies which binds to an epitope in a region from the 524th amino acid to the 580th amino acid of human glypican 3, preferably a region from the 524th amino acid to the 563rd amino

acid, more preferably a region from the 537th amino acid to the 563rd amino acid, further more preferably a region from the 544th amino acid to the 553rd amino acid, particularly preferably a region from the 546th amino acid to the 551st amino acid.

Another preferred embodiment according to the present invention is an antibody that recognizes a region from the 524th amino acid to the 563rd amino acid of human glypican 3 and does not recognize a region from the 537th amino acid to the 563rd amino acid.

A further preferred embodiment according to the present invention is an antibody that recognizes a region from the 537th amino acid to the 563rd amino acid of human glypican 3 and does not recognize a region from the 550th amino acid to the 563rd amino acid.

The analysis of an epitope recognized by an antibody can be carried out by a method known to those skilled in the art, for example, by Western blotting described in Examples below.

The antibody that recognizes the above-mentioned regions as an epitope can be obtained by a method known to those skilled in the art. For example, it can be obtained by preparing a peptide containing an amino acid sequence of a target region based on an amino acid sequence of human glypican 3 and preparing an antibody with the use of the peptide as an immunogene, or by preparing an antibody by a usual method and

determining an epitope that the obtained antibody recognizes, and then selecting an antibody that recognizes the target epitope.

A preferred example of the anti-glypican 3 antibody of the present invention is an antibody having a high ADCC activity or an antibody having a high CDC activity to a cell that expresses glypican 3.

The phrase "a high ADCC activity" or "a high CDC activity" as used herein means that the antibody of the invention has a higher ADCC activity or a higher CDC activity than that of a known anti-glypican 3 antibody. Known glypican 3 antibodies include, for example, M3C11 and M1E07 described in International Patent Application WO 2004/22739.

The ADCC activity or the CDC activity can be measured by a method known to those skilled in the art. For example, it can be measured by the chromium release test. Specific conditions of the chromium release test for measuring the ADCC activity are not particularly limited, however, for example, it can be measured using the conditions described in the Examples below.

Examples of the cells that express glypican 3 include, for example, a hepatoma cell line such as HepG2, a CHO cell line having a gene encoding glypican 3 incorporated therein and the like. To measure the ADCC activity, it is preferred to use a HepG2 cell line, and to measure the CDC activity, it

is preferred to use a recombinant CHO cell line that expresses GPC3. The recombinant CHO cell line that expresses GPC3 may be prepared by any method, however, it can be prepared by, for example, the method described in the Examples below.

In the case where the anti-glypican 3 antibody is used as an anticancer agent, it is preferred that it has an ADCC activity at the same level as that of an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 62 and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 73 (GC33). In the case where the anti-glypican 3 antibody is used as an anticancer agent, it is preferred that it has a CDC activity at the same level as that of an antibody containing a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 62 and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 73 (GC33).

Further, the present invention includes an antibody having a high binding activity to glypican 3.

In the present invention, the binding activity of the antibody to glypican 3 can be measured by using a method known to those skilled in the art. For example, it can be measured by utilizing the surface plasmon resonance with BIAcore. Specifically, a glypican 3 protein is immobilized on a sensor chip to react with an antibody, and the interaction between the antibody and glypican 3 can be calculated as a reaction

rate constant from the measurement value. In addition, with regard to the evaluation of the binding activity, an enzyme linked immunosorbent assay (ELISA), an enzyme immunoassay (EIA), a radioimmunoassay (RIA) or a fluorescent antibody technique can be used. For example, in the case where an enzyme immunoassay is used, a sample containing an antibody to be tested, for example, a culture supernatant of a cell producing an antibody to be tested or a purified antibody is added to a plate which has been coated with an antigen to which the antibody to be tested binds. Then, a secondary antibody labeled with an enzyme such as alkaline phosphatase is added, and the plate is incubated and washed. Then, an enzyme substrate such as p- nitrophenyl phosphate is added and the absorbance is measured, whereby an antigen binding activity can be evaluated. The upper limit of the binding activity is not particularly limited. However, for example, the upper limit can be defined within the range which is technically possible by those skilled in the art. It will be appreciated that the range which is technically possible will be expanded by the advancement of technology.

Further, in the present invention, an amino acid to be deamidated or an amino acid adjacent to an amino acid to be deamidated may be replaced with another amino acid for the purpose of, for example, suppressing deamidation to increase the stability of the antibody. The amino acid to be deamidated

includes, asparagine and glutamine, preferably asparagine. An amino acid adjacent to asparagine is not particularly limited and may be any amino acid. It is known that an asparagine-glycine sequence is particularly susceptible to deamidation, thus, glycine is preferred as the amino acid adjacent to asparagine. An amino acid used for replacement is not particularly limited and may be any amino acid other than asparagine and glutamine. Preferred is an amino acid other than valine and proline. Therefore, in the present invention, in the case where the antibody is deamidated, it is preferred to replace the amino acid with an amino acid other than asparagine, glutamine, valine and proline. Suppression of deamidation by amino acid replacement can be carried out with reference to, for example, International Patent Application WO 03/057881. In the case where amino acid replacement is carried out for the purpose of suppression of deamidation, it is preferred that the antigen binding activity before replacement is maintained.

Another embodiment of stabilization of the antibody includes replacement of glutamic acid with another amino acid. In addition, in the present invention, it was found that, in the case where the 6th amino acid of the heavy chain of an antibody is glutamic acid, the antibody can be significantly stabilized by replacing the glutamic acid with glutamine. Accordingly, the present invention also relates to a method

of stabilizing an antibody by replacing the glutamic acid at the 6th position of the heavy chain of the antibody with glutamine. The amino acid numbering of the antibody is known to those skilled in the art (e.g., Kabat, E. A. et al., "Sequences of Proteins of Immunological Interest", US Dept. Health and Human Services 1983).

#### Method of preparing antibody

The antibody that binds to glypican 3 can be prepared by a method known to those skilled in the art. For example, a monoclonal antibody-producing hybridoma can be prepared as follows basically using a known technique. That is, the hybridoma can be prepared by immunizing a mammal in accordance with a usual immunization method using a glypican 3 protein or a cell that expresses glypican 3 as a sensitizing antigen. The thus obtained immunocyte is fused with a known parent cell by a usual cell fusion method, and then selecting a monoclonal antibody-producing cell by a usual screening method.

Specifically, a monoclonal antibody can be prepared as follows. First, a glypican 3 protein is obtained based on the glypican 3 gene/amino acid sequence shown in SEQ ID NOs: 3 and 4, which is used as a sensitizing antigen to obtain an antibody. More specifically, the gene sequence encoding glypican 3 is inserted into a known expression vector system, and an appropriate host cell is transformed with the vector, and then



a target human glypican 3 protein is purified by a known method from the host cell or the culture supernatant.

Subsequently, this purified glypican 3 protein is used as a sensitizing antigen. Alternatively, a partial peptide of glypican 3 can be used as a sensitizing antigen. In this case, the partial peptide can also be obtained by chemical synthesis according to the amino acid sequence of human glypican 3.

The epitope on a glypican 3 molecule which is recognized by the anti-glypican 3 antibody of the present invention is not limited to a particular epitope. The anti-glypican 3 antibody may recognize any epitope, as long as the epitope is present on a glypican 3 molecule. Accordingly, any fragment can also be used as an antigen for producing the anti-glypican 3 antibody of the present invention, as long as it contains an epitope that is present on a glypican 3 molecule.

A mammal to be immunized with a sensitizing antigen is not particularly limited, but it is preferably selected in view of compatibility with a parent cell to be used for cell fusion. For example, rodents such as mice, rats and hamsters, rabbits or monkeys are generally used.

Immunization of an animal with a sensitizing antigen is carried out in accordance with a known method. For example, immunization is carried out by a general method in which a mammal is injected intraperitoneally or subcutaneously with

a sensitizing antigen. Specifically, a sensitizing antigen is diluted with or suspended in an appropriate amount of PBS (Phosphate-Buffered Saline), physiological saline or the like, an appropriate amount of a standard adjuvant such as a Freund's complete adjuvant is mixed with the product if necessary, and then the solution is emulsified and is administered to a mammal several times every 4 to 21 days. In addition, an appropriate carrier can also be used upon immunization with a sensitizing antigen.

A mammal is immunized as described above, and then an increased level of a target antibody in the serum is confirmed. Subsequently, immunocytes are collected from the mammal, and then subjected to cell fusion. A particularly preferred immunocyte is a splenocyte.

As a parent partner cell to be fused with the above-mentioned immunocyte, a mammalian myeloma cell is used. Examples of a cell line of the myeloma cell that is preferably used herein include various known cell lines such as P3 (P3x63 Ag8.653) (J. Immunol. (1979) 123, 1548-1550), P3x63 Ag8U.1 (Current Topics in Microbiology and Immunology (1978) 81, 1-7), NS-1 (Kohler. G. and Milstein, C. Eur. J. Immunol. (1976) 6, 511-519), MPC-11 (Margulies. D. H. et al., Cell (1976) 8, 405-415), SP2/0 (Shulman, M. et al., Nature (1978) 276, 269-270), FO (de St. Groth, S. F. et al., J. Immunol. Methods (1980) 35, 1-21), S194 (Trowbridge, I. S. J. Exp. Med. (1978)

148, 313-323) and R210 (Galfre, G. et al., Nature (1979) 277, 131-133).

Cell fusion of the above-mentioned immunocytes with myeloma cells can be basically carried out in accordance with a known method, for example, the method of Kohler and Milstein et al. (Kohler, G. and Milstein, C., Methods Enzymol. (1981) 73, 3-46).

More specifically, the above-mentioned cell fusion is carried out in a normal nutrition culture solution in the presence of, for example, a cell-fusion accelerator. As the cell-fusion accelerator, for example, polyethylene glycol (PEG), a hemagglutinating virus of Japan (HVJ) is used. If desired, an adjuvant such as dimethylsulfoxide can be added to further enhance the fusion efficiency.

The ratio of immunocytes to myeloma cells may be appropriately selected. For example, it is preferred that the number of immunocytes is 1 to 10 times greater than that of myeloma cells. The culture solution to be used for the above-mentioned cell fusion include, for example, a RPMI1640 culture solution or a MEM culture solution which is suitable for the growth of the above-mentioned myeloma cell line, or another normal culture solution which is used for this type of cell culture. Moreover, a serum supplement such as fetal calf serum (FCS) can be used in combination therewith.

Cell fusion is carried out as follows. Predetermined

amounts of the above-mentioned immunocytes and myeloma cells are mixed well in the above-mentioned culture solution, a PEG (e.g., with an average molecular weight of approximately 1000 to 6000) solution (a general concentration of 30 to 60% (w/v)), which had been pre-heated at approximately 37°C, is added, and then the solution is mixed, whereby a target fusion cell (hybridoma) is formed. Subsequently, an appropriate culture solution is added successively, and then a step of removing the supernatant by centrifugation is repeated to remove a reagent for cell fusion or the like that is unfavorable for the growth of the hybridoma.

The thus obtained hybridoma is selected by culturing the hybridoma in a standard selective culture solution such as a HAT culture solution (a culture solution containing hypoxanthine, aminopterin and thymidine). Cultivation in the above-mentioned HAT culture solution is continued for a time period sufficient for the cells (unfused cells) other than the target hybridoma to die (normally, several days to several weeks). Subsequently, a standard limiting dilution method is conducted to screen for and monoclonal of hybridoma that produces a target antibody.

In addition to the method of immunizing a non-human animal with an antigen to obtain hybridoma, a desired human antibody having a binding activity to glypican 3 can also be obtained by sensitizing a human lymphocyte with glypican 3 in

vitro, and allowing the sensitized lymphocyte to fuse with a human-derived myeloma cell having a permanent division potential (see JP-B-1-59878). In another method, glypican 3 antigen is administered to a transgenic animal having all the repertoires of human antibody genes to obtain anti-glypican 3 antibody-producing cells, which are then immortalized, and a human antibody for glypican 3 may be obtained from the immortalized anti-glypican 3 antibody-producing cells (see International Patent Applications WO 94/25585, WO 93/12227, WO 92/03918 and WO 94/02602).

The thus prepared hybridoma that produce a monoclonal antibody can be passage-cultured in a standard culture solution, or can be stored for a long period in liquid nitrogen.

One example of a method employed to obtain a monoclonal antibody from the hybridoma involves culturing the hybridoma and obtaining a monoclonal antibody from the culture supernatant in accordance with a standard method. Another method involves administering the hybridoma to a mammal that is compatible with the hybridoma to allow it to proliferate, and obtaining a monoclonal antibody from the ascites. The former method is suitable to obtain an antibody of high purity, while the latter method is suitable for the mass production of antibodies.

It is also possible to prepare a recombinant antibody by cloning the antibody gene from the hybridoma, incorporating

the gene into an appropriate vector, introducing the vector into a host, and then allowing the host to produce the recombinant antibody by a genetic engineering technique (e.g., see Vandamme, A. M. et al., Eur. J. Biochem. (1990) 192, 767-775, 1990).

Specifically, mRNA encoding the variable (V) region of an anti-glypican 3 antibody is isolated from a hybridoma producing the anti-glypican 3 antibody. mRNA is isolated by a known method such as a guanidine ultracentrifugal method (Chirgwin, J. M. et al., Biochemistry (1979) 18, 5294-5299) or an AGPC method (Chomczynski, P. et al., Anal. Biochem. (1987) 162, 156-159), and total RNA is prepared, and then target mRNA is prepared using an mRNA Purification Kit (Pharmacia) or the like. In addition, mRNA can also be directly prepared using a QuickPrep mRNA Purification Kit (Pharmacia).

The cDNA of the antibody V region is synthesized using a reverse transcriptase from the thus obtained mRNA. cDNA may be synthesized using an AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (SEIKAGAKU CORPORATION) or the like. In addition, for example, a 5'-Ampli FINDER RACE Kit (Clontech), the 5'-RACE method using PCR (Frohman, M. A. et al., Proc. Natl. Acad. Sci. USA (1988) 85, 8998-9002, Belyavsky, A. et al., Nucleic Acids Res. (1989) 17, 2919-2932) can be employed for synthesizing and amplifying cDNA.

A target DNA fragment is purified from the thus obtained

PCR product, and then ligated to a vector DNA. A recombinant vector is prepared from the product, and then the vector is introduced into *E. coli* or the like, and a colony is selected, thereby preparing a desired recombinant vector. The nucleotide sequence of the target DNA is then determined by a known method such as a dideoxynucleotide chain termination method.

After DNA encoding the V region of the target anti-glypican 3 antibody is obtained, this DNA is incorporated into an expression vector containing DNA encoding the constant region (C region) of the target antibody.

To produce the anti-glypican 3 antibody used in the present invention, the antibody gene is incorporated into an expression vector so that the gene is expressed under the regulation of the gene expression control region including, for example, an enhancer and a promoter. Next, a host cell is transformed with the expression vector, thereby allowing the host to express the antibody.

An antibody gene can be expressed by incorporating a polynucleotide encoding the H chain or a polynucleotide encoding the L chain separately into an expression vector, and then simultaneously transforming a host cell with the vectors, or by incorporating polynucleotides encoding the H chain and the L chain into a single expression vector, and then transforming a host cell with the vector (see International

Patent Application WO 94/11523).

### Polynucleotide

In another aspect, the present invention provides a polynucleotide encoding a heavy chain variable region or a light chain variable region of the antibody of the present invention. Preferably, the polynucleotide of the present invention has a nucleotide sequence described in any of SEQ ID NOs: 11-21, 33-43, 55-59, 65-70 and 77-83. In addition, a polynucleotide that is hybridized to the above-mentioned polynucleotide under stringent conditions and encodes an antibody having an activity equivalent to that of the antibody of the present invention is also within the scope of the present invention.

The polynucleotide of the present invention is not particularly limited as long as it encodes the antibody of the present invention. It is a polymer composed of a plurality of nucleotides, such as deoxyribonucleic acids (DNA) or ribonucleic acids (RNA). It may contain a base other than a naturally occurring base. The polynucleotide of the present invention can be used for producing an antibody by a genetic engineering technique. In addition, the polynucleotide of the present invention can be used as a probe to screen for an antibody having a function equivalent to that of the antibody of the present invention. That is, a polynucleotide encoding



the antibody of the present invention or a partial fragment thereof may be used as a probe to obtain DNA that is hybridized to the polynucleotide under stringent conditions and encodes an antibody having an activity equivalent to that of the antibody of the present invention by techniques such as a hybridization technique, a gene amplification technique (e.g., PCR). Such DNA is also included in the polynucleotide of the present invention.

The hybridization technique (Sambrook, J. et al., Molecular Cloning 2nd ed., 9.47-9.58, Cold Spring Harbor Lab. Press, 1989) is well known to those skilled in the art. Examples of the hybridization conditions include, for example, low stringent conditions. The low stringent conditions are, for example, the conditions of 42°C, 0.1x SSC and 0.1% SDS, preferably the conditions of 50°C, 0.1x SSC and 0.1% SDS when washing is performed after hybridization. More preferred examples of the hybridization conditions include, for example, high stringent conditions. The high stringent conditions are, for example, the conditions of 65°C, 5x SSC and 0.1% SDS. Under these conditions, it can be expected that a polynucleotide having a higher homology can be efficiently obtained under higher temperature. Incidentally, there are plural factors that affect the stringency of hybridization, such as temperature and the concentration of salt, and those skilled in the art can achieve a similar stringency by appropriately

selecting these factors.

An antibody functionally equivalent to the antibody of the present invention encoded by a polynucleotide obtained by such a hybridization technique and a gene amplification technique usually has a high homology with the antibody in terms of the amino acid sequence. The antibody of the present invention also includes an antibody that is functionally equivalent to the antibody of the present invention and has a high homology with the amino acid sequence of the antibody. A high homology means generally at least 50% or higher identity, preferably 75% or higher identity, more preferably 85% or higher identity, and further more preferably 95% or higher identity at the amino acid level. To determine the homology of polypeptides, the algorithm described in the literature (Wilbur, W. J. and Lipman, D. J., Proc. Natl. Acad. Sci. USA (1983) 80, 726-730) may be employed.

The present invention also provides a vector containing the polynucleotide of the present invention. Such a vector can be used for preparing the antibody of the present invention. As for the vector of the present invention, in the case where *E. coli* is used as a host, for example, it is not particularly limited as long as it has "ori" for use in amplification in *E. coli* to produce and amplify the vector in a large amount in *E. coli* (e.g., JM109, DH5 $\alpha$ , HB101 or XL1Blue), and has a marker gene for selecting a transformed *E. coli* (e.g., a drug

resistance gene that can be identified by a drug such as ampicillin, tetracycline, kanamycin or chloramphenicol). Examples of the vector include M13-series vectors, pUC-series vectors, pBR322, pBluescript, pCR-Script and the like. In addition, pGEM-T, pDIRECT, and pT7 can also be used for subcloning and extracting cDNA as well as the vectors described above.

As the vector of the present invention, an expression vector is particularly useful. For example, an expression vector to be expressed in *E. coli* should have the above characteristics to be amplified in *E. coli*. In addition, in the case where *E. coli*, such as JM109, DH5 $\alpha$ , HB101, or XL1-Blue is used as a host cell, it is indispensable that the vector should have a promoter, for example, lacZ promoter (Ward et al., *Nature* (1989) 341, 544-546; *FASEB J.* (1992) 6, 2422-2427), araB promoter (Better et al., *Science* (1988) 240, 1041-1043), T7 promoter or the like, that can efficiently express the desired product in *E. coli*. Examples of such a vector include pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), pEGFP, pET (in this case, the host is preferably BL21 which expresses T7 RNA polymerase) and the like, as well as the vectors described above.

In addition, the vector may also contain a signal sequence for polypeptide secretion. As for the signal sequence for protein secretion, in the case where a polypeptide

is produced in the periplasm of *E. coli*, the *pelB* signal sequence (Lei S. P. et al., *J. Bacteriol* (1987) 169, 4379) can be used. Introduction of the vector into a host cell can be carried out by using, for example, the calcium chloride method and the electroporation method.

In addition to *E. coli*, for example, expression vectors derived from mammals (e.g., pcDNA3 (Invitrogen) and pEGF-BOS (Nucleic Acids Res. (1990) 18(17), p5322), pEF and pCDM8), expression vectors derived from insect cells (e.g., "Bac-to-BAC baculovirus expression system" (GIBCO BRL) and pBacPAK8), expression vectors derived from plants (e.g., pMH1 and pMH2), expression vectors derived from animal viruses (e.g., pHSV, pMV and pAdexLcw), expression vectors derived from retroviruses (e.g., pZIPneo), expression vectors derived from yeast (e.g., "Pichia Expression Kit" (Invitrogen), pNV11 and SP-Q01), and expression vectors derived from *Bacillus subtilis* (e.g., pPL608 and pKTH50) can be used as the vector of the present invention.

For the purpose of expressing the vector in an animal cell such as a CHO cell, a COS cell, an NIH3T3 cell or the like, it is indispensable for the vector to have a promoter required for expression in a cell such as SV40 promoter (Mulligan et al., *Nature* (1979) 277, 108), MMTV-LTR promoter, EF1 $\alpha$  promoter (Mizushima et al., *Nucleic Acids Res.* (1990) 18, 5322), CMV promoter or the like, and more preferably to have a marker gene

(such as a drug resistance gene that can be identified by a drug such as neomycin or G418) for selecting transformation into the cell. Examples of the vector having such characteristics include, for example, pMAM, pDR2, pBK-RSV, pBK-CMV, pOPRSV and pOP13.

Further, for the purpose of stably expressing a gene and, at the same time, amplifying the gene copy numbers in the cell, a vector (e.g., pCHOI, etc.) having the DHFR gene is introduced into the CHO cell deficient in the nucleic acid synthetic pathway to complement the deficiency and is amplified with methotrexate (MTX). In addition, for the purpose of transient expression of a gene, transformation is effected with a vector (such as pcD) having the origin of replication for SV40 using a COS cell having on the chromosome a gene that expresses the SV40 T antigen. As the origin of replication, the one derived from a polyoma virus, an adenovirus, a bovine papilloma virus (BPV) and the like can also be used. Further, for the amplification of gene copy numbers in the host cell system, the expression vector can include, as a selectable marker, the aminoglycoside transferase (APH) gene, the thymidine kinase (TK) gene, E. coli xanthine guaninephosphoribosyl transferase (Ecogpt) gene, the dihydrofolate reductase (dhfr) gene and the like.

To prepare the antibody of the present invention, the vector is introduced into a host cell. The host cell into which

the vector is introduced is not particularly limited, but includes, for example, E. coli or any of various animal cells. For example, the host cell can be used as a production system for the production or expression of the antibody of the present invention. As for the production system of polypeptide preparation, there are an in vitro production system and an in vivo production system. In vitro production system include a production system which employs eukaryotic cells and a production system which employs prokaryotic cells.

In the case where the eukaryotic cell is used, for example, an animal cell, a plant cell or a fungal cell can be used. Known animal cells include a mammalian cell such as a CHO cell (J. Exp. Med. (1995) 108, 945), a COS cell, a 3T3 cell, a myeloma cell, a baby hamster kidney (BHK) cell, a HeLa cell and a Vero cell, an amphibian cell such as a Xenopus oocyte (Valle, et al., Nature (1981) 291, 358-340), or an insect cell such as Sf9, Sf21, and Tn5. In the present invention, CHO-DG44, CHO-DXB11, a COS7 cell, a BHK cell are preferably used. Among the animal cells, for the purpose of performing a large amount of expression, a CHO cell is particularly preferred. Introduction of the vector into the host cell can be carried out by, for example, the calcium phosphate method, the DEAE-dextran method, the cationic ribosome DOTAP (Boehringer Mannheim), the electroporation method, the lipofection method or the like.

As for the plant cell, for example, a cell derived from *Nicotiana tabacum* is known as a protein production system, which may be subjected to callus culture. Examples of the fungal cells include yeast such as the genus *Saccharomyces*, more specifically *Saccharomyces cerevistae* and *Saccharomyces pombe*, and filamentous fungi such as the genus *Aspergillus*, more specifically *Aspergillus niger*.

In the case where the prokaryotic cell is used, production system using a bacterial cell may be employed. Examples of the bacterial cells include *Escherichia coli* (*E. coli*) such as JM109, DH5 $\alpha$  and HB101, and *Bacillus subtilis*.

#### Preparation of recombinant antibody

The antibody of the present invention can be prepared by culturing the above-mentioned host cells. The antibody can be obtained by culturing in vitro a cell transformed with a desired polynucleotide. Cultivation can be carried out in accordance with a known method. Culture media for animal cells include, for example, DMEM, MEM, RPMI 1640, and IMDM. A serum supplement such as FBS or fetal calf serum (FCS) may be used in combination, or serum-free medium can be used. The pH during the cultivation is preferably about 6 to 8. Cultivation is usually carried out at about 30 to 40°C for about 15 to 200 hours with, as needed, medium change, aeration, and agitation.

On the other hand, systems for producing a polypeptide

in vivo include, for example, a production system which employs an animal and a production system which employs a plant. The target polynucleotide is introduced into such an animal or a plant, and the polypeptide is produced in the body of the animal or the plant and recovered. The term "host cell" as used herein encompasses such an animal and a plant.

When the animal is used, production systems employing a mammal or an insect are available. As the mammal, goats, pigs, sheep, mice and cattle can be used (Vicki Glaser, SPECTRUM Biotechnology Applications, 1993). A transgenic animal can also be used as a mammal.

For example, the target polynucleotide is prepared as a fusion gene with a gene encoding a polypeptide which is inherently produced in the milk such as goat  $\beta$  casein. Then, the DNA fragment containing this fusion gene is injected into a goat embryo, and the embryo is transplanted into a female goat. The target antibody can be obtained from the milk produced by the transgenic goat borne to the goat which received the embryo or the offspring thereof. To increase the amount of milk containing the antibody produced by the transgenic goat, hormone may be given to the transgenic goat as needed. (Ebert, K. M. et al., Bio/Technology (1994) 12, 699-702).

In addition, as an insect, for example, a silkworm can be used. In the case where a silkworm is used, a silkworm is infected with a baculovirus into which the polynucleotide



encoding the target antibody has been inserted. The target antibody can be obtained from the body fluid of the silkworm (Susumu, M. et al., Nature (1985) 315, 592-594).

In the case where a plant is used, for example, tobacco can be used. In the case where tobacco is used, a polynucleotide encoding the target antibody is inserted into an expression vector for a plant, for example pMON 530, and then the vector is introduced into a bacterium such as *Agrobacterium tumefaciens*. Then, tobacco such as *Nicotiana tabacum* is infected with the bacterium, whereby the target antibody can be obtained from the leaves of the tobacco (Julian, K. -C. Ma et al., Eur. J. Immunol. (1994) 24, 131-138).

The thus obtained antibody can be isolated from the inside or the outside (culture medium, etc.) of the host cell and then can be purified to a substantially pure and uniform antibody. Separation and purification of the antibody may be carried out by a separation and a purification method usually used in purification of polypeptides. For example, polypeptides can be separated and purified by any methods including chromatography columns, filtration, ultrafiltration, salting-out, solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, dialysis, recrystallization, and a combination thereof.

Examples of the chromatography include, for example,

affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel-filtration, reverse phase chromatography, adsorption chromatography (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press, 1996). These chromatographies can be carried out using a liquid phase chromatography such as HPLC and FPLC. Examples of a column to be used for affinity chromatography include a protein A column or a protein G column. One example of the protein A column is Hyper D, POROS, Sepharose F. F. (Pharmacia).

Further, before or after purification of the antibody, the antibody can be modified or peptides can be partially removed as needed by allowing a suitable protein-modifying enzyme to act on. The protein-modifying enzyme for this purpose include, for example, trypsin, chymotrypsin, lysyl endopeptidase, protein kinase, glucosidase.

#### Diagnostic method

In another aspect, the present invention provides a method of diagnosing a disease such as cancer by detecting GPC3 protein in a test sample with the use of the antibody of the present invention.

The detection used herein includes quantitative detection and non-quantitative detection. The non-

quantitative detection include, for example, determination of merely whether or not GPC3 protein is present, determination of whether or not a specific amount or more of GPC3 protein is present, determination for comparison of the amount of GPC3 protein with that of another sample (e.g., a control sample). The quantitative detection includes determination of the concentration of GPC3 protein, determination of the amount of GPC3 protein.

The test sample is not particularly limited as long as it is a sample that may possibly contain GPC3 protein, however, preferred is a sample collected from the body of a living organism such as a mammal, and more preferred is a sample collected from human. Specific examples of the test sample may include, for example, blood, interstitial fluid, plasma, extravascular fluid, cerebral fluid, joint fluid, pleural fluid, serum, lymph fluid, saliva, preferably blood, serum and plasma. In addition, a sample obtained from the test sample such as culture solution of cells collected from the body of the living organism is also included in the test sample of the present invention.

The cancer to be diagnosed is not particularly limited, and specific examples may include liver cancer, pancreatic cancer, lung cancer, colon cancer, mammary cancer, prostate cancer, leukemia and lymphoma, preferably liver cancer. GPC3 to be detected is not particularly limited, and may be either

full-length GPC3 or a fragment thereof. In the case where a fragment of GPC3 is detected, it may be either the N-terminal fragment or the C-terminal fragment, however, the N-terminal fragment is preferred. In addition, the GPC3 protein may also be a heparan sulfate-added GPC3 or a GPC3 core protein.

The method of detecting GPC3 protein contained in a test sample is not particularly limited, however, detection is preferably performed by an immunological method with the use of an anti-GPC3 antibody. Examples of the immunological method include, for example, a radioimmunoassay, an enzyme immunoassay, a fluorescence immunoassay, a luminescence immunoassay, immunoprecipitation, a turbidimetric immunoassay. Preferred is an enzyme immunoassay, and particularly preferred is an enzyme-linked immunosorbent assay (ELISA) (e.g., a sandwich ELISA). The above-mentioned immunological method such as an ELISA can be carried out by a method known to those skilled in the art.

A general detection method with the use of an anti-GPC3 antibody comprises immobilizing an anti-GPC3 antibody on a support, adding a test sample thereto, incubating the support to allow the anti-GPC3 antibody and GPC3 protein to bind to each other, washing the support, and detecting the GPC3 protein binding to the support via the anti-GPC3 antibody to detect GPC3 protein in a test sample.

The binding between the anti-GPC3 antibody and the GPC3

protein is generally carried out in a buffer. Buffers used in the invention include, for example, a phosphate buffer, a Tris buffer. Incubation is carried out under the conditions generally employed, for example, at 4°C to room temperature for 1 hour to 24 hours. The washing after incubation can be carried out by any method as long as it does not inhibit the binding between the GPC3 protein and the anti-GPC3 antibody, using for example a buffer containing a surfactant such as Tween 20.

In the method of detecting GPC3 protein of the present invention, a control sample may be provided in addition to a test sample to be tested for GPC3 protein. The control samples include a negative control sample that does not contain GPC3 protein and a positive control sample that contains GPC3 protein. In this case, it is possible to detect GPC3 protein in the test sample by comparing the result obtained with the negative control sample that does not contain GPC3 protein with the result obtained with the positive control sample that contains GPC3 protein. It is also possible to quantitatively detect GPC3 protein contained in the test sample by obtaining the detection results of the control samples and the test sample as numerical values, and comparing these numerical values.

One preferred embodiment of detecting GPC3 protein binding to the support via an anti-GPC3 antibody is a method using an anti-GPC3 antibody labeled with a detectable label.

For example, GPC3 protein may be detected by contacting the test sample with an anti-GPC3 antibody immobilized on the support, washing the support, and then detecting GPC3 with the use of the labeled antibody that specifically binds to GPC3 protein.

The labeling of an anti-GPC3 antibody can be carried out by a generally known method. Examples of the detectable label known to those skilled in the art include a fluorescent dye, an enzyme, a coenzyme, a chemiluminescent substance or a radioactive substance. Specific examples may include radioisotopes ( $^{32}\text{P}$ ,  $^{14}\text{C}$ ,  $^{125}\text{I}$ ,  $^3\text{H}$ ,  $^{131}\text{I}$  and the like), fluorescein, rhodamine, dansyl chloride, umbelliferone, luciferase, peroxidase, alkaline phosphatase,  $\beta$ -galactosidase,  $\beta$ -glucosidase, horseradish peroxidase, glucoamylase, lysozyme, saccharide oxidase, microperoxidase, biotin and the like. In the case where biotin is used as a detectable label, it is preferred that a biotin-labeled antibody is added, and then avidin conjugated to an enzyme such as alkaline phosphatase is further added.

Specifically, a solution containing an anti-GPC3 antibody is added to a support such as a plate to allow the anti-GPC3 antibody to be immobilized. After washing, the plate is blocked with, for example, BSA in order to prevent the nonspecific binding of a protein. The plate is washed again, and then the test sample is added to the plate. After being

incubated, the plate is washed, and then the labeled anti-GPC3 antibody is added. After being incubated appropriately, the plate is washed, and then the labeled anti-GPC3 antibody remaining on the plate is detected. The detection of the protein can be carried out by a method known to those skilled in the art. For example, in the case where the antibody is labeled with a radioactive substance, the protein may be detected by liquid scintillation or the RIA method. In the case where the antibody is labeled with an enzyme, the protein may be detected by adding a substrate and detecting an enzymatic change of the substrate such as color development with an absorbance reader. In the case where the antibody is labeled with a fluorescent substance, the protein may be detected with the use of a fluorometer.

A particularly preferred embodiment of the method of detecting GPC3 protein of the present invention is a method using an anti-GPC3 antibody labeled with biotin and avidin. Specifically, a solution containing an anti-GPC3 antibody is added to a support such as a plate to allow the anti-GPC3 antibody to be immobilized thereon. After washing, the plate is blocked with, for example, BSA in order to prevent the nonspecific binding of a protein. The plate is washed again, and then the test sample is added to the plate. After being incubated, the plate is washed, and then the biotin-labeled anti-GPC3 antibody is added. After being incubated

appropriately, the plate is washed, and then avidin conjugated to an enzyme such as alkaline phosphatase or peroxidase is added. After being incubated, the plate is washed, and then a substrate of the enzyme conjugated to avidin is added. Then, GPC3 protein is detected by means of the enzymatic change of the substrate as an indicator.

Another embodiment of the method of detecting GPC3 protein of the present invention is a method using a primary antibody that specifically binds to GPC3 protein and a secondary antibody that specifically binds to the primary antibody. For example, the test sample is brought into contact with an anti-GPC3 antibody immobilized on the support, the support is incubated and washed, and the bound GPC3 protein after washing is detected with a primary anti-GPC3 antibody and a secondary antibody that specifically binds to the primary antibody. In this case, the secondary antibody is preferably labeled with a detectable label.

Specifically, a solution containing an anti-GPC3 antibody is added to a support such as a plate to allow the anti-GPC3 antibody to be immobilized thereon. After washing, the plate is blocked with, for example, BSA in order to prevent the nonspecific binding of a protein. The plate is washed again, and then the test sample is added to the plate. After being incubated, the plate is washed, and then a primary anti-GPC3 antibody is added. After being incubated appropriately, the



plate is washed, and then a secondary antibody that specifically binds to the primary antibody is added. After being incubated appropriately, the plate is washed, and then the secondary antibody remaining on the plate is detected. The detection of the secondary antibody can be carried out by the above-mentioned method.

#### Pharmaceutical composition

In another aspect, the present invention provides a pharmaceutical composition containing the antibody of the present invention. The pharmaceutical composition containing the antibody of the present invention is useful in the treatment and/or prevention of a disease associated with cell proliferation such as cancer, and particularly it is useful in the treatment and/or prevention of liver cancer. In the case where the antibody of the present invention is used as a pharmaceutical composition, the antibody can be formulated into a dosage form by a method known to those skilled in the art. For example, the pharmaceutical composition can be used parenterally in the form of an injection of a sterile solution or a suspension with water or another pharmaceutically acceptable solution. For example, the antibody can be formulated into a dosage form by appropriately mixing it with a pharmaceutically acceptable carrier or solvent, such as sterile water, physiological saline, a plant-oil, an

emulsifier, a suspension, a surfactant, a stabilizer, a flavor, an excipient, a vehicle, a preservative, a binder to prepare a unit dosage form required for generally accepted Drug Implementation. The amount of active ingredients in these preparations is selected to allow for administration of a suitable dosage within the indicated range.

A sterile composition for injection can be formulated by using a vehicle such as distilled water for injection in accordance with the general Drug Implementation.

Examples of the aqueous solution for injection include, for example, physiological saline, glucose, and other isotonic liquids including adjuvants, such as D-sorbitol, D-mannnose, D-mannitol and sodium chloride. They can be used in combination with a suitable solubilizer, such as an alcohol, specifically ethanol, a polyalcohol such as propylene glycol and polyethylene glycol, and a non-ionic surfactant such as Polysorbate 80 (TM) and HCO-50.

Sesame oil or soybean oil can be used as a oleaginous liquid and may be used in combination with benzyl benzoate or benzyl alcohol as a solubilizer. It may be formulated with a buffer such as a phosphate buffer or a sodium acetate buffer, a pain-killer such as procaine hydrochloride, a stabilizer such as benzyl alcohol or phenol, or an antioxidant. The prepared injection is generally filled into a suitable ampule.

The method of administration is preferably parenteral,

and specific examples thereof include injection, transnasal administration, transpulmonary administration, transdermal administration and the like. The injection formulation may be administered systemically or topically by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection or the like.

The method of administration can be appropriately selected according to the age and the symptoms of a patient. For example, one dose of the pharmaceutical composition containing the antibody or the polynucleotide encoding the antibody can be selected from the range of 0.0001 mg to 1,000 mg per kg of body weight. Alternatively, for example, the dose can be selected from the range of 0.001 mg to 100,000 mg/body per patient, although it is not always limited to these numerical values. The dose and the method of administration vary according to the body weight, the age and the symptoms of a patient, and are appropriately selected by those skilled in the art.

#### EXAMPLE

The present invention will be described in more detail with reference to Examples below. However, the present invention is not limited to these Examples.

##### Example 1

### cdNA cloning of human glypican 3 (GPC3)

A full-length cDNA encoding human GPC3 was amplified by PCR reaction with an Advantage 2 kit (CLONTECH) using 1st stranded cDNA prepared by a usual method from a colon cancer cell line, Caco2, as a template. More specifically, 50  $\mu$ L of a reaction mixture containing 2  $\mu$ L of cDNA derived from Caco2, 1  $\mu$ L of a sense primer (GATATC-ATGGCCGGGACCGTGCGCACCGCGT: SEQ ID NO: 1), 1  $\mu$ L of an antisense primer (GCTAGC-TCAGTGCACCAGGAAGAAGAAGCAC: SEQ ID NO: 2), 5  $\mu$ L of Advantage 2 10x PCR buffer, 8  $\mu$ L of dNTP mix (1.25 mM) and 1.0  $\mu$ L of Advantage polymerase Mix was subjected to 35 cycles consisting of 94°C for 1 minute, 63°C for 30 seconds and 68°C for 3 minutes. The amplified product from the PCR reaction was inserted into a TA vector, pGEM-T Easy, using pGEM-T Easy Vector System I (Promega). The sequence was confirmed by using an ABI 3100 DNA sequencer. In this way, a cDNA encoding full-length human GPC3 was isolated. The sequence shown in SEQ ID NO: 3 indicates the nucleotide sequence of human GPC3 gene and the sequence shown in SEQ ID NO: 4 indicates the amino acid sequence of human GPC3 protein.

### Example 2

#### Preparation of soluble form of GPC3

As immunoprotein for the generation of an anti-GPC3 antibody, a soluble form of GPC protein was prepared, in which

a hydrophobic region at the C-terminal side (564-580 amino acids) was deleted.

By using the full-length human GPC3 cDNA as a template, a PCR reaction was carried out using an antisense primer (ATA GAA TTC CAC CAT GGC CGG GAC CGT GCG C: SEQ ID NO: 5) and a sense primer, to which an EcoRI recognition sequence and a Kozak sequence were added, (ATA GGA TCC CTT CAG CGG GGA ATG AAC GTT C: SEQ ID NO: 6). The obtained PCR fragment (1711 bp) was cloned into pCXND2-Flag. The pCXND2-Flag was designed to express a Flag-tagged protein by inserting the region for DHFR gene expression of pCHOI (Hirata et al., FEBS letter 1994; 356; 244-248) into the HindIII site of pCXN2 (Niwa et al., Gene 1991; 108; 193-199) and adding a Flag tag sequence to the downstream of the multicloning site. The constructed expression plasmid DNA was introduced into a CHO cell line, DXB11, and a CHO cell line highly expressing the soluble form of GPC3 was obtained by selection with 500 µg/mL Geneticin. The large-scale cultivation of the CHO cell line highly expressing the soluble form of GPC3 was carried out using a 1700-cm<sup>2</sup> roller bottle, and the culture supernatant was recovered for the antibody purification. The culture supernatant was applied to a DEAE sepharose Fast Flow column (Amersham) and, after washing, the antibody was eluted with a buffer containing 500 mM NaCl, and affinity purified using Anti-Flag M2 agarose affinity gel (SIGMA). The elution was carried out with 200 µg/mL FLAG

peptide. After the eluate was concentrated with Centriprep-10 (Millipore), FLAG peptide was removed by gel filtration using Superdex 200 HR 10/30 (Amersham). Lastly, the filtrate was concentrated using a DEAE sepharose Fast Flow column and eluted with PBS (containing 500 mM NaCl) without Tween 20 to effect buffer exchange.

### Example 3

#### Preparation of soluble form of GPC3 core protein

GPC3 is modified by heparan sulfate to become a macromolecule. To eliminate an antibody against heparan sulfate in a screening for an anti-GPC3 antibody, a soluble form of GPC3 core protein that had a point mutation in the heparan sulfate-binding site was prepared and used in the screening.

Using the above-mentioned soluble form of GPC3 (1-563) as a template, a cDNA in which Ser residues at the 495th and 509th positions were replaced with Ala was prepared by the assembly PCR method, in which primers were designed to add His tag to the C-terminus. The obtained cDNA was cloned into pCXND3 vector. The pCXND3 was constructed by inserting the DHFR gene expressing region of pCHOI in the HindIII site of pCXN2. The constructed expression plasmid DNA was introduced into DXB11 cell line and a CHO cell line highly expressing a soluble form of GPC3 core protein was obtained by selection with 500  $\mu$ g/mL

Geneticin.

The large-scale cultivation was carried out using a 1700-cm<sup>2</sup> roller bottle and the culture supernatant was recovered for antibody purification. The culture supernatant was applied to a Q sepharose Fast Flow column (Amersham). After washing, the antibody was eluted with a phosphate buffer containing 500 mM NaCl, and affinity purified using a Chelating sepharose Fast Flow column (Amersham). The antibody was eluted with a gradient of 10 to 150 mM imidazole. Lastly, the eluate was concentrated using a Q sepharose Fast Flow column and, eluted with a phosphate buffer containing 500 mM NaCl.

SDS polyacrylamide gel electrophoresis under reducing conditions showed three bands of 70 kDa, 40 kDa and 30 kDa. The result of amino acid sequencing using an ABI492 protein sequencer (Applied Biosystems) indicated that the 30 kDa band corresponded to the amino acid sequence of the 359th and its downstream or the 375th and its downstream of GPC3, suggesting that GPC3 was cleaved between Arg358 and Ser359 or between Lys374 and Val375, hence, it was separated into 40 kDa of the N-terminal fragment and 30 kDa of the C-terminal fragment.

#### Example 4

##### Preparation of CHO cell line expressing full-length human GPC3

To obtain a cell line for evaluating a binding activity using flow cytometry, a CHO cell line expressing full-length

GPC3 was established.

Ten microgram of a full-length human GPC3 gene expression vector and 60  $\mu$ L of SuperFect (QIAGEN) were mixed. After a complex was formed, gene introduction was carried out by adding it to a CHO cell line, DXB11. After a 24-hour cultivation in a CO<sub>2</sub> incubator, selection was started using  $\alpha$ MEM (GIBCO BRL) containing Geneticin at a final concentration of 0.5 mg/mL and 10% FBS. The resulting Geneticin-resistant colonies were collected and cell cloning was carried out by the limiting dilution method. Each cell clone was solubilized and the expression of full-length human GPC3 was confirmed by Western blotting using an anti-GPC3 antibody. In this way, a stably expressing cell line was obtained.

#### Example 5

##### Evaluation of binding activity by ELISA

The soluble form of GPC3 core protein was diluted to 1  $\mu$ g/mL with a coating buffer (0.1 mol/L NaHCO<sub>3</sub> (pH 9.6), 0.02% (w/v) NaN<sub>3</sub>) and added to an immunoplate and left at 4°C overnight to coat the plate. After the plate was blocked with a dilution buffer (50 mmol/L Tris-HCl (pH 8.1), 1 mmol/L MgCl<sub>2</sub>, 150 mmol/L NaCl, 0.05% (v/v) Tween 20, 0.02% (w/v) NaN<sub>3</sub>, 1% (w/v) BSA), an anti-GPC3 antibody was added and left at room temperature for 1 hour. After washing with a rinse buffer (0.05% (v/v) Tween 20, PBS), an anti-mouse IgG antibody (ZYMED) labeled with



alkaline phosphatase was added and left at room temperature for 1 hour. After washing with the rinse buffer, SIGMA 104 (SIGMA) diluted to 1 mg/mL with a substrate buffer (50 mmol/L NaHCO<sub>3</sub> (pH 9.8), 10 mmol/L MgCl<sub>2</sub>) was added and left at room temperature for 1 hour for color development. Then the absorbance (at 405 nm, reference wavelength of 655 nm) was measured using a Benchmark Plus (BIO-RAD).

#### Example 6

##### Immunization with soluble form of GPC3 and selection of hybridoma

Since human GPC3 and mouse GPC3 show a high homology of 94% at the amino acid level, it was considered difficult to obtain an anti-GPC3 antibody if a normal mouse was immunized. Therefore, an autoimmune disease mouse, MRL/MpJUmCrj-lpr/lpr mouse, (hereinafter referred to as MRL/lpr mouse, purchased from Charles River Japan, Inc.) was used as an immunized animal. Immunization was started at the age of 7 weeks or 8 weeks. For the first immunization, a soluble form of GPC3 was prepared at 100 µg/head and emulsified using Freund's complete adjuvant (FCA, Becton Dickinson) and subcutaneously administered. Two weeks later, a soluble form of GPC3 was prepared at 50 µg/head and emulsified using Freund's incomplete adjuvant (FIA, Becton Dickinson) and subcutaneously administered. After that, an additional immunization was carried out every other week for

5 times in total. To two of the immunized mice, a soluble form of GPC3 was diluted with PBS to 50 µg/head, and then administered intravenously via the tail as the final immunization. On the forth day after the final immunization, the spleen was excised to obtain a spleen cell, which was mixed with a mouse myeloma cell, P3-X63Ag8U1 (P3U1, purchased from ATCC), at a ratio of 2:1. Cell fusion was carried out by gradually adding PEG 1500 (Roche Diagnostic). RPMI 1640 medium (GIBCO BRL) was carefully added to dilute PEG 1500, and after PEG 1500 was removed by centrifugation, the cells were suspended in RPMI 1640 medium containing 10% FBS and inoculated into a 96-well culture plate at 100 µL/well. On the next day, RPMI 1640 medium containing 10% FBS, 1x HAT media supplement (SIGMA) and 0.5x BM-Condimed H1 Hybridoma cloning supplement (Roche Diagnostic) (hereinafter referred to as HAT medium) was added at 100 µL/well. After 2, 3 and 5 days, half of the culture solution was replaced with the HAT medium. After 7 days, screening was carried out using the culture supernatant. The screening was carried out by an ELISA using an immunoplate coated with the soluble form of GPC3 core protein. A positive clone was monocloned by the limiting dilution method. As a result, 11 clones of antibodies (M3C11, M13B3, M1E7, M3B8, M11F1, L9G11, M19B11, M6B1, M18D4, M5B9 and M10D2) that have a strong binding activity against GPC3 were obtained.

#### Example 7

##### Isotype determination and purification of anti-GPC3 antibody

Isotype was determined by an antigen-dependent ELISA using an Immunopure Monoclonal Antibody Isotyping Kit I (PIERCE). The purification of antibodies was carried out as follows. The culture supernatant of hybridoma cultured with the HAT medium supplemented with FBS (Ultra low IgG) (GIBCO BRL) was adsorbed to Hi Trap ProteinG HP (Amersham), and washed with a binding buffer (20 mM sodium phosphate (pH 7.0)). The antibody was eluted with an elution buffer (0.1 M glycine-HCl (pH 2.7)). The eluate was immediately neutralized with a neutralization buffer (1 M Tris-HCl (pH 9.0)), and dialyzed against PBS for day and night for buffer exchange.

#### Example 8

##### Evaluation of binding activity by ELISA

In order to conveniently evaluate the binding activity of the anti-GPC3 antibody thus obtained, concentration-dependent binding of the antibody was detected against an immunoplate containing the soluble form of GPC3 core protein immobilized thereon. A 3-fold dilution series (12 dilutions in total) of the purified antibody at a concentration of 10 µg/mL was added, and an anti-mouse IgG antibody was added as the secondary antibody. Color development was carried out using SIGMA 104. Since the degree of color development varies

depending on the color development time, data measured precisely after 1 hour was analyzed. Every antibody showed a concentration-dependent color development. The correlation between the concentration of antibody and the degree of color development was plotted and an approximate curve was obtained by using an analyzing software, GraphPad Prism. Its EC50 value was determined as the index of the binding activity. EC50 values for all clones are shown in Fig. 16.

#### Example 9

##### Evaluation of binding activity by flow cytometry

Cells were dissociated with 1 mM EDTA pH 8.0 (GIBCO)/PBS and suspended in FACS buffer (1% FBS/PBS) at  $1 \times 10^6$  cells/mL. The suspension was dispensed to a Multiscreen-HV Filter Plate (Milipore) at 100  $\mu$ L/well and the supernatant was removed by centrifugation. An anti-GPC3 antibody diluted to an appropriate concentration was added and reacted on ice for 30 minutes. The cells were washed once with FACS buffer and an FITC-labeled anti-mouse IgG antibody was added and reacted on ice for 30 minutes. After the reaction, the cells were centrifuged at 500 rpm for 1 minute, and the supernatant was removed. The cells were suspended in 400  $\mu$ L of FACS buffer and subjected to flow cytometry. EPICS ELITE ESP (Beckman Coulter) was used as a flow cytometer. A gate was set on the living cell population with the histogram of forward scatter

and side scatter. As shown in Fig. 1, an anti-GPC3 antibody (M3C11, M11F1) bound strongly to the CHO cell expressing GPC3 and did not bind to the parent CHO cell, indicating that the antibody specifically binds to GPC3 presented on the cell membrane. In addition, the antibody showed the binding activity to a hepatoma cell line, HepG2 (purchased from ATCC) and HuH-7 (purchased from Health Science Research Resources Bank), suggesting that the antibody may specifically recognize hepatoma. The binding activity of the clones derived from the mouse immunized with a soluble form of GPC3 measured by flow cytometry is shown in Fig. 16, where the X-mode values of histogram at the concentration of antibody of 5  $\mu\text{g/mL}$  are indicated.

#### Example 10

##### Epitope classification by competitive ELISA

The obtained antibodies were classified according to the epitopes by a competitive ELISA. The antibodies were biotinylated using a Biotin Labeling Kit (Roche). The soluble form of GPC3 core protein was diluted to 1  $\mu\text{g/mL}$  with the coating buffer and added to a plate at 100  $\mu\text{L/well}$  and stored at 4°C overnight to coat the plate. On the next day, 200  $\mu\text{L}$  of the substrate buffer was added for blocking. The plate was left at 4°C overnight or longer and an anti-GPC3 antibody was added to the plate at 100  $\mu\text{L/well}$  and reacted at room temperature

for 1 hour. After that, without washing of the plate, 10  $\mu$ L of 10  $\mu$ g/mL biotin-labeled anti-GPC3 antibody was added and further reacted for 1 hour. The plate was washed with 300  $\mu$ L/well of the rinse buffer for 3 times. AP-streptavidin conjugate (ZYMED) was diluted to 1000-fold with the dilution buffer and added at 100  $\mu$ L/well and reacted at room temperature for 1 hour. The plate was washed with 300  $\mu$ L/well of the rinse buffer for 5 times. SIGMA 104 was diluted to 1 mg/mL with the substrate buffer and added at 100  $\mu$ L/well. After incubating for 1 hour at room temperature, the absorbance (at 405 nm, reference wavelength of 655 nm) was measured.

The results of the competitive ELISA are shown in Fig. 2. As for the antibody that competitively inhibited the binding of the biotinylated antibody by 50% or more, it was considered that its epitopes are located close together in the three-dimensional conformation. As a result of classification according to the competitive inhibition pattern of color development against the binding of the 8 types of biotinylated antibodies, the 11 clones derived from the mouse immunized with a soluble form of GPC3 were classified into 5 groups (a, b, c, d and e) (Fig. 16).

#### Example 11

##### Epitope classification by Western blotting

The soluble form of GPC3 core protein was applied to a

10% SDS-PAGE mini (TEFCO) and electrophoresed under reducing conditions. It was transferred to Immobilon-P (Millipore) using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BIO-RAD). After the membrane was briefly washed with TBS-T (0.05% Tween 20, TBS), it was shaken in TBS-T containing 5% skim milk for 1 hour. The membrane was shaken in TBS-T for about 10 minutes, then each anti-GPC3 antibody diluted with TBS-T containing 1% skim milk was added and the membrane was shaken for 1 hour. The membrane was washed with TBS-T and shaken in a solution of HRP-anti-mouse IgG antibody (Amersham) diluted with TBS-T containing 1% skim milk for 1 hour, and then washed with TBS-T. Color development was carried out using ECL-Plus (Amersham) and detected using Hyperfilm ECL (Amersham).

As shown in Fig. 3, L9G11 was determined to be an antibody binding to the N-terminal side because it bound to the band of about 40 kDa. M3C11 was determined to be an antibody binding to the C-terminal side because it bound to the band of about 30 kDa. All the antibodies belonging to c, d or e group based on the competitive ELISA bound to the N-terminal side, and all those belonging to a or b groups bound to the C-terminal side (Fig. 16). L9G11 had higher detection sensitivity in Western blotting than the other antibodies that bind to the N-terminal side, suggesting that this antibody is a useful for detecting the N-terminal fragment by Western blotting.

## Example 12

### Detection of secreted form of GPC3

Since it was found that GPC3 is cleaved at the 358th amino acid residue or the 374th amino acid residue, the inventors hypothesized that a secreted form of GPC3 is secreted into the blood of a patient with liver cancer. Therefore, a GPC3 sandwich ELISA system was constructed in order to detect a secretory form of GPC3.

An immunoplate was coated with an anti-GPC3 antibody at 10 µg/mL and blocked by the substrate buffer. After the immunoplate was stored for several hours at room temperature or overnight at 4°C, the culture supernatant of HepG2 was added and incubated for 1 hour at room temperature. The immunoplate was washed with 300 µL/well of the rinse buffer for 3 times, and a biotin-labeled anti-GPC3 antibody diluted to 10 µg/mL was added and incubated for 1 hour at room temperature. The immunoplate was washed with 300 µL/well of the rinse buffer for 3 times, and AP-streptavidin was added and incubated for 1 hour at room temperature. The immunoplate was washed with 300 µL/well of the rinse buffer for 5 times. Color development was carried out using AMPAK (DAKO) in accordance with the attached protocol and the absorbance was measured using a microplate reader. The antibodies binding to the N-terminal side (M6B1, M19B11 and M18D4) and those binding to the



C-terminal side (M3C11, M13B3 and M3B8) were combined to construct five sandwich ELISA systems. Each of these combinations showed an equivalent sensitivity in the standard curve using the secreted form of GPC3. These systems were evaluated using the culture supernatant of HepG2. The secreted form of GPC3 was detected at a high concentration of about 1 µg/mL with a combination of the antibodies binding to the N-terminal side (Fig. 4). The concentration detected with a combination of the antibodies binding to the C-terminal side was low, suggesting that the N-terminal fragment was dominantly present in the secreted form of GPC3.

Subsequently, the culture supernatant of HepG2 was immunoprecipitated using an anti-GPC3 antibody to detect the secreted form of GPC3. In the case where M10D2 that binds to the N-terminal fragment was used, the secreted form of GPC3 of 40 kDa was detected (Fig. 5). On the other hand, in the case where M1E7 that binds to the C-terminal fragment was used, the secreted form of GPC3 was not detected. The immunoprecipitation test was carried out for all the obtained GPC3 antibodies. Every antibody binding to the N-terminal fragment strongly detected the secreted form of GPC3, while the secreted form of GPC3 was not detected or was weakly detected with the use of the antibodies binding to the C-terminal fragment (Fig. 16). The antibody that can detect the secreted form of GPC3 by immunoprecipitation is expected

to be useful as an antibody for diagnosing hepatoma. In addition, the antibody that can hardly detect the secreted form of GPC3 is expected to be useful in the development of a therapeutic antibody having an ADCC activity and a CDC activity, because such an antibody may migrate to the hepatoma lesion without being trapped in the secreted form of GPC3 present in the blood.

#### Example 13

##### Cloning of variable region of anti-GPC3 antibody

A variable region of the anti-GPC3 antibody was amplified by the RT-PCR method using the total RNA extracted from an anti-GPC3 antibody-producing hybridoma. The total RNA was extracted from  $1 \times 10^7$  cells of the hybridoma with the use of RNeasy Plant Mini Kits (QIAGEN). By using 1  $\mu$ g of the total RNA, the 5'-terminal gene fragment was amplified with the use of a SMART RACE cDNA Amplification Kit (CLONTECH) and any of the following synthetic oligonucleotides:

a synthetic oligonucleotide MHC-IgG1 complementary to the sequence of a mouse IgG1 constant region:

GGG CCA GTG GAT AGACAG ATG (SEQ ID NO: 7);

a synthetic oligonucleotide MHC-IgG2a complementary to the sequence of a mouse IgG2a constant region:

CAG GGG CCA GTG GAT AGA CCG ATG (SEQ ID NO: 8);

a synthetic oligonucleotide MHC-IgG2b complementary to the

sequence of a mouse IgG2b constant region:

CAG GGG CCA GTG GAT AGA CTG ATG (SEQ ID NO: 9); and

a synthetic oligonucleotide kappa complementary to the sequence of a mouse kappa chain constant region:

GCT CAC TGG ATG GTG GGA AGA TG (SEQ ID NO: 10).

A reverse transcription reaction was carried out at 42°C for 1 hour and 30 minutes. The PCR mixture (50 µL) contained 5 µL of 10x Advantage 2 PCR buffer, 5 µL of 10x Universal Primer A Mix, 0.2 mM dNTPs (dATP, dGTP, dCTP and dTTP), 1 µL of Advantage 2 Polymerase Mix (all from CLONTECH), 2.5 µL of the reverse transcription reaction product and 10 pmol of the synthetic oligonucleotide MHC-IgG1, MHC-IgG2a, MHC-IgG2b or kappa. PCR was carried out with 5 cycles consisting of 94°C for 30 seconds, 94°C for 5 seconds and 72°C for 3 minutes, 5 cycles consisting of 94°C for 5 seconds, 70°C for 10 seconds and 72°C for 3 minutes, and 25 cycles consisting of 94°C for 5 seconds, 68°C for 10 seconds and 72°C for 3 minutes. Lastly, the reaction product was heated at 72°C for 7 minutes. Each PCR product was purified from the agarose gel using a QIAquick Gel Extraction Kit (QIAGEN), cloned into pGEM-T Easy vector (Promega), and the nucleotide sequence was determined.

The nucleotide sequences of the H chain variable regions of M3C11, M13B3, M1E7, M3B8, M11F1, M19B11, M6B1, M18D4, M5B9, M10D2 and L9G11 are shown in SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 and 21, respectively, the amino acid

sequences thereof are shown in SEQ ID NOs: 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32, respectively. The nucleotide sequences of the L chain variable regions thereof are shown in SEQ ID NOs: 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 and 43, respectively, and the amino acid sequences thereof are shown in SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50, 51, 52, 53 and 54, respectively.

#### Example 14

##### Epitope classification using GST-fusion protein

To carry out a detail analysis of the epitopes for the antibodies binding to the C-terminal fragment, fusion proteins of successively shortened C-terminal peptides of GPC3 with GST, namely GC-1 (from Ser495 to Lys563), GC-2 (from Gly510 to Lys563), GC-3 (from Ala524 to Lys563), GC-4 (from Gly537 to Lys563) and GC-5 (from Ser550 to Lys563) were prepared. The C-terminal region of GPC3 was cloned into pGEX-4T-3 (Amersham) to construct a plasmid DNA in which the C-terminal region of GPC3 is ligated to the C-terminal side of GST. The plasmid DNA was introduced into DH5 $\alpha$ , whereby a transformant was obtained. Then, IPTG was added at 1 mM to a culture of the transformant in the logarithmic growth phase to induce the expression of a GST-fusion protein. The bacterial cells were collected after 2 hours cultivation. The cells were homogenized by sonication, and centrifuged at 35,000 rpm for

30 minutes with XL-80 ultracentrifuge (Beckman, 70.1 Ti rotor). Then, the culture supernatant was recovered and purified with GST Purification Modules (Amersham). The thus purified GST-fusion proteins were separated by SDS-PAGE under reducing conditions, and Western blotting was carried out with the anti-GPC3 antibodies (Fig. 6). M3C11 and M1E7 detected GC-1 and GC-2, while they did not detect GC-3, GC-4 and GC-5, indicating that the epitopes of these antibodies are contained in the region of GC-2, and that the region of GC-3 is not sufficient. M3B8 and M11F1 detected GC-1 GC-2, GC-3 and GC-4, while they did not detect GC-5, indicating that the epitopes of these antibodies are contained in the region of GC-4, and that the region of GC-5 is not sufficient. The minimum region of the GST-fusion protein to which each antibody can bind is listed in the column headed "Western blotting" of Fig. 16.

#### Example 15

##### Preparation of anti-GPC3 mouse-human chimeric antibody

The sequences of the H chain and the L chain variable regions of the anti-GPC3 antibodies were ligated to the sequences of a human IgG1 and a kappa chain constant regions. PCR was carried out by using a synthetic oligonucleotide, which is complementary to the 5'-terminal nucleotide sequence of the H chain variable region of each antibody and has a Kozak sequence, and a synthetic oligonucleotide, which is

complementary to the 3'-terminal nucleotide sequence and has a NheI site. The obtained PCR product was cloned into pB-CH vector in which a human IgG1 constant region was inserted into pBluescript KS(+) vector (Toyobo). The mouse H chain variable region and the human H chain ( $\gamma$ 1 chain) constant region are ligated via the NheI site. The prepared H chain gene fragment was cloned into an expression vector, pCXND3. On the other hand, PCR was carried out by using a synthetic oligonucleotide, which is complementary to the 5'-terminal nucleotide sequence of the L chain variable region of each antibody and has a Kozak sequence, and a synthetic oligonucleotide, which is complementary to the 3'-terminal nucleotide sequence and has a BsiWI site. The obtained PCR product was cloned into pB-CL vector in which the human kappa chain constant region was inserted into pBluescript KS(+) vector (Toyobo). The human L chain variable region and the constant region are ligated via the BsiWI site. The prepared L chain gene fragment was cloned into an expression vector, pUCAG. This pUCAG vector was obtained by cloning a 2.6 kbp fragment obtained by digesting pCXN (Niwa et al., Gene 1991; 108: 193-200) with a restriction enzyme BamHI into the BamHI site of pUC19 vector (Toyobo).

To prepare an expression vector for an anti-GPC3 mouse-human chimeric antibody, a gene fragment was obtained by digesting the pUCAG vector containing the L chain gene fragment with a restriction enzyme HindIII (Takara Shuzo), and

cloned into the HindIII site of the pCXND3 containing the H chain gene. This plasmid will express a neomycin-resistance gene, DHFR gene and an anti-GPC3 mouse-human chimeric antibody in an animal cell.

A CHO cell line (DG44 cell line) stably expressing the antibody was prepared as follows. The gene was introduced into the cells by the electroporation method using Gene Pulser II (Bio-Rad). A mixture obtained by mixing 25  $\mu$ g of the expression vector for each anti-GPC3 mouse-human chimeric antibody and 0.75 mL of a solution of CHO cells suspended in PBS ( $1 \times 10^7$  cell/mL) was cooled on ice for 10 minutes, and transferred to a cuvette. Then, a pulse was applied at 1.5 kV and a capacitance of 25  $\mu$ FD. After a 10-minute recovery period at room temperature, the electroporated cells were suspended in 40 mL of CHO-S-SFM II medium (Invitrogen) containing 1x HT supplement (Invitrogen). The suspension was diluted to 50-fold with the same medium, and dispensed to a 96-well culture plate at 100  $\mu$ L/well. After a 24-hour culture in a CO<sub>2</sub> incubator (5% CO<sub>2</sub>), Geneticin (Invitrogen) was added at 0.5 mg/mL and the cells were cultured for 2 weeks. The culture supernatant was taken from the well having a Geneticin resistant transformed cell colony and the amount of IgG was measured by the concentration determination method described below. A high-producing cell line was successively expanded to obtain a cell line that stably expresses an anti-GPC3 mouse-human chimeric antibody. The

cell line was cultured at a large-scale and the culture supernatant was collected. The purification of each anti-GPC3 mouse-human chimeric antibody was carried out using Hi Trap ProteinG HP (Amersham).

#### Example 16

##### Measurement of complement-dependent cytotoxicity activity (CDC activity)

##### 16.1 Preparation of human albumin veronal buffer (HAVB)

In milli-Q water, 12.75 g of NaCl (highest grade, Wako Pure Chemicals), 0.5625 g of Na-Barbital (highest grade, Wako Pure Chemicals) and 0.8625 g of Barbital (highest grade, Wako Pure Chemicals) were dissolved to a final volume of 200 mL and autoclaved at 121°C for 20 minutes. Then, 100 mL of autoclaved hot milli-Q water was added. The pH was 7.43 (recommended pH: 7.5). The solution was used as a 5x veronal buffer. In 50 mL of milli-Q water, 0.2205 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (highest grade, Junsei Chemical) was dissolved to a final concentration of 0.03 mol/L, which was used as a  $\text{CaCl}_2$  solution. In 50 mL of milli-Q water, 1.0165 g of  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (highest grade, Junsei Chemical) was dissolved to a final concentration of 0.1 mol/L, which was used as a  $\text{MgCl}_2$  solution. In milli-Q water, 100 mL of the 5x veronal buffer, 4 mL of human serum albumin (25% Buminat (registered trademark), the concentration of human serum albumin: 250 mg/mL, Baxter Healthcare), 2.5 mL of the  $\text{CaCl}_2$  solution, 2.5 mL of



the  $\text{MgCl}_2$  solution, 0.1 g of KCl (highest grade, Junsei Chemical) 0.5 g of glucose (D(+)-glucose, anhydrous glucose, highest grade, Wako Pure Chemicals) were dissolved to a final volume of 500 mL, which was used as HAVB. After filter sterilization, the HAVB was stored at a preset temperature of 5°C.

#### 16.2 Preparation of target cell

The CHO cell expressing full-length human GPC3 prepared in Example 4 was cultured in  $\alpha$ -MEM medium containing nucleic acid (+) (GIBCO) supplemented with 10% FBS and 0.5 mg/mL Geneticin (GIBCO). The cells were dissociated from the dish using a cell dissociation buffer (Invitrogen Corp), dispensed to each well of a 96-well flat-bottomed plate (Falcon) at  $1 \times 10^4$  cells/well, and cultured for 3 days. After the cultivation, 5.55 MBq of chromium-51 was added, and the cells were cultured in a 5% carbon dioxide gas incubator at 37°C for 1 hour. These cells were washed with HAVB twice, and 50  $\mu\text{L}$  of HAVB was added and used as a target cell.

#### 16.3 Chromium release test (CDC activity)

Each chimeric antibody was diluted with HAVB to make a 40  $\mu\text{g}/\text{mL}$  antibody solution. To the target cell, 50  $\mu\text{L}$  of each antibody solution was added, and left on ice for 15 minutes. Subsequently, to each well, 100  $\mu\text{L}$  of the human serum from the

peripheral blood of a healthy volunteer, which had been diluted with HAVB, was added to a final concentration of 25% (the final concentration of antibody: 10 µg/mL), and left in a 5% carbon dioxide gas incubator at 37°C for 90 minutes. After the plate was centrifuged, 100 µL of the supernatant was collected from each well, the radioactivity was measured using a gamma counter. The specific chromium release rate was obtained by the following formula.

$$\text{Specific chromium release rate (\%)} = (A-C) \times 100 / (B-C)$$

"A" represents the radioactivity (cpm) in each well, "B" represents the mean value of the radioactivities (cpm) in the wells in which 100 µL of 2% NP-40 aqueous solution (Nonidet P-40, Code No. 252-23, Nacalai Tesque) and 50 µL of HAVB were added to the target cell, and "C" represents the mean value of the radioactivities (cpm) in the wells in which 150 µL of HAVB was added to the target cell. The test was carried out in triplicate and the mean value and the standard deviation were calculated for CDC activity (%).

The results are shown in Fig. 7. Among 9 types of the anti-GPC3 chimeric antibodies, M3B8 and M11F1, which are an antibody recognizing the C-terminal side, showed a strong CDC activity against the CHO cell expressing GPC3, however, the CDC activity was not observed in the other antibodies. M3B8 and M11F1 belong to the group called "b" based on the competitive ELISA, and an important epitope for showing a

strong CDC activity could be found.

#### Example 17

#### Measurement of ADCC activity using PBMC derived from human peripheral blood

##### 17.1 Preparation of human PBMC solution

The heparinized peripheral blood obtained from a healthy volunteer was diluted to 2-fold with PBS(-), and overlaid on Ficoll-Paque TM PLUS (Amersham). After centrifugation at 500 x g for 30 minutes at 20°C, the middle layer, which is the mononuclear leukocyte fraction, was collected. The cells were washed 3 times, suspended in 10% FBS/RPMI and used as a human PBMC solution.

##### 17.2 Preparation of target cell

The HepG2 cells cultured in 10% FBS/RPMI 1640 medium were dissociated from the dish using Trypsin-EDTA (Invitrogen), dispensed to each well of a 96-well U-bottomed plate (Falcon) at  $1 \times 10^4$  cells/well, and cultured for 2 days. The CHO cell expressing full-length human GPC3 prepared in Example 4 was cultured in  $\alpha$ -MEM nucleic acids (+) medium (GIBCO) supplemented with 10% FBS and 0.5 mg/mL Geneticin (GIBCO). The cells were dissociated from the dish using a cell dissociation buffer (Invitrogen Corp), dispensed to each well of a 96-well flat-bottomed plate (Falcon) at  $1 \times 10^4$  cells/well, and cultured

for 3 days. Chromium-51 (5.55 MBq) was added to each cell and the cells were cultured in a 5% carbon dioxide gas incubator at 37°C for 1 hour. These cells were washed with the medium once, and 50 µL of 10% FBS/RPMI 1640 medium was added and used as a target cell.

### 17.3 Chromium release test (ADCC activity)

To the target cell, 50 µL of an antibody solution prepared at different concentrations was added, and reacted on ice for 15 minutes. Subsequently, 100 µL of the human PBMC solution was added at  $5 \times 10^5$  cells/well, and cells were cultured in a 5% carbon dioxide gas incubator at 37°C for 4 hours. After the cultivation, the plate was centrifuged, and the radioactivity in 100 µL of the culture supernatant was measured using a gamma counter. The specific chromium release rate was obtained by the following formula.

$$\text{specific chromium release rate (\%)} = (A-C) \times 100 / (B-C)$$

"A" represents the mean value of the radioactivities (cpm) in each well, "B" represents the mean value of the radioactivities (cpm) in the wells in which 100 µL of 2% NP-40 aqueous solution (Nonidet P-40, Code No. 252-23, Nacalai Tesque) and 50 µL of 10% FBS/RPMI medium were added to the target cell, and "C" represents the mean value of the radioactivities (cpm) in the wells in which 150 µL of 10% FBS/RPMI medium was added to the target cell. The test was carried out in triplicate and the

mean value and the standard deviation were calculated for ADCC activity (%). The results are shown in Fig. 8. Among 9 types of the anti-GPC3 chimeric antibodies, the antibodies recognizing the C-terminal side had a tendency of showing a strong ADCC activity.

#### Example 18

##### Immunization with GC-3 and selection of hybridoma

Among the obtained anti-GPC3 antibodies, only M11F1 and M3B8 showed a strong CDC activity, indicating that the CDC activity is epitope dependent. To obtain an antibody having both ADCC activity and CDC activity, a GST-fusion protein containing the epitope for M11F1 and M3B8, referred to as GC-3, was used for immunization. A large amount of GC-3 was purified by the above-mentioned method. The buffer was changed to PBS by gel filtration using Superdex 75 (Amersham). The obtained product was used as immunoprotein. using three Balb/c mice (purchased from Charles River Japan, Inc.) and three MRL/lpr mice were immunized with GC-3 in accordance with the above-mentioned method. For the first immunization, GC-3 was prepared at 100  $\mu$ g/head and emulsified using FCA, which was subcutaneously administered. Two weeks later, GC-3 was prepared at 50  $\mu$ g/head and emulsified using FIA, which was subcutaneously administered. After the fifth immunization, the final immunization (50  $\mu$ g/head) was carried out for all

mice by intravenously administering the immunoprotein via the tail. After cell fusion, hybridoma were screened by an ELISA using an immunoplate coated with the soluble form of GPC3 core protein. A positive clone was monocloned by the limiting dilution method. As a result, 5 clones of antibodies (GC199, GC202, GC33, GC179 and GC194) that have a strong binding activity against GPC3 were obtained.

The antibody was purified from the culture supernatant of the hybridoma using Hi Trap proteinG HP, and analyzed in accordance with the above-mentioned method. The EC50 value was calculated by an ELISA using an immunoplate coated with the soluble form of GPC3 core protein, and the X-mode value of histogram at 5  $\mu\text{g/mL}$  was measured by flow cytometry (Fig. 17). According to the epitope classification by a competitive ELISA, the antibodies were classified into the group b (GC199, GC202 and GC33) and a new epitope group f (GC179 and GC194). The epitope classification using the GST-fusion proteins indicated that GC199, GC202 and GC33 detected GC-1, GC-2, GC-3 and GC-4, but did not detect GC-5, suggesting that the epitopes for these antibodies are contained in the region of GC-4 in the same manner as the epitopes for M11F1 and M3B8, and that the region of GC-5 is not sufficient. On the other hand, GC179 and GC194 detected GC-1, GC-2 and GC-3, but did not detect GC-4 and GC-5, suggesting that the epitopes for these antibodies are contained in the region of GC-3, and that the region of

GC-4 is not sufficient. The minimum region of the GST-fusion protein to which each antibody can bind is listed in the column headed "Western blotting" of Fig. 17.

The H chain and the L chain variable regions of GC199, GC202, GC33, GC179 and GC194 were cloned in accordance with the above-mentioned method, and their sequences were determined. As for the L chain of GC194, 2 types of sequences were cloned. The nucleotide sequences of the H chain variable regions of GC199, GC202, GC33, GC179 and GC194 are shown in SEQ ID NOs: 55, 56, 57, 58 and 59, respectively, and the amino acid sequences thereof are shown in SEQ ID NOs: 60, 61, 62, 63 and 64, respectively. The nucleotide sequences of the L chain variable regions of GC199, GC202, GC33, GC179, GC194(1) and GC194(2) are shown in SEQ ID NOs: 65, 66, 67, 68, 69 and 70 respectively, and the amino acid sequences thereof are shown in SEQ ID NOs: 71, 72, 73, 74, 75 and 76, respectively.

Further, these amino acid sequences were examined for homology by comparing with the database of the amino acid sequences of known antibodies, whereby their CDR regions were determined as follows.

Antibody	CDR	Amino Acid Sequence	SEQ ID NO
M13B3 (H)	CDR1	NYAMS	103
	CDR2	AINNNGDDTYYLDTVKD	104
	CDR3	QGGAY	105
M3B8 (H)	CDR1	TYGMGVG	106
	CDR2	NIWWYDAKYNSDLKS	107

	CDR3	MGLAWFAY	108
M11F1 (H)	CDR1	IYGMGVG	109
	CDR2	NIWWNDDKYNSALKS	110
	CDR3	IGYFYFDY	111
M5B9 (H)	CDR1	GYWMH	112
	CDR2	AIYPGNSDTNYNQKFKG	113
	CDR3	SGDLTGGLAY	114
M6B1 (H)	CDR1	SYAMS	115
	CDR2	AINSNGGTTYYPDTMKD	116
	CDR3	HNGGYENYGWFAY	117
M10D2 (H)	CDR1	SYWMH	118
	CDR2	EIDPSDSYTYYNQKFRG	119
	CDR3	SNLGDGHYRFPAPFY	120
L9G11 (H)	CDR1	SYWMH	118
	CDR2	TIDPSDSETHYNLQFKD	121
	CDR3	GAFYSSYSYWAWFAY	122
GC33 (H)	CDR1	DYEMH	123
	CDR2	ALDPKTGDTAYSQKFKG	124
	CDR3	FYSYTY	125
GC179 (H)	CDR1	INAMN	126
	CDR2	RIRSESNNYATYYGDSVKD	127
	CDR3	EVTTSFAY	128
GC194 (H)	CDR1	ASAMN	129
	CDR2	RIRSKSNNYAIYYADSVKD	130
	CDR3	DPGYGPNPWFAY	131
GC199 (H)	CDR1	DYSMH	132
	CDR2	WINTETGEPTYADDFKG	133
	CDR3	LY	134
GC202 (H)	CDR1	TYGMGVG	106
	CDR2	NIWWHDDKYNSALKS	135
	CDR3	IAPRYNKYEGFFAF	136



M13B3 (L)	CDR1	KSSQSLDSDGKTYLN	137
	CDR2	LVSKLDS	138
	CDR3	WQGFHFPLT	139
M3B8 (L)	CDR1	KASQDINNYLS	140
	CDR2	RANRLVD	141
	CDR3	LQCDEFPPWT	142
M11F1 (L)	CDR1	RSSQSLVHSNGNTYLH	143
	CDR2	KVSNRFS	144
	CDR3	SQSTHVPWT	145
M5B9 (L)	CDR1	RSSKSLHLSNGITYLY	146
	CDR2	QMSNLAS	147
	CDR3	AQNLELPYT	148
M6B1 (L)	CDR1	KASQDINKNII	149
	CDR2	YTSTLQP	150
	CDR3	LQYDNLPR	151
M10D2 (L)	CDR1	RASHSISNFLH	152
	CDR2	YASQSI	153
	CDR3	QQSNIWSLT	154
L9G11 (L)	CDR1	RASESVEYYGTSLMQ	155
	CDR2	GASNVES	156
	CDR3	QQRKVPYT	157
GC33 (L)	CDR1	RSSQSLVHSNGNTYLH	143
	CDR2	KVSNRFS	144
	CDR3	SQNTHVPPT	158
GC179 (L)	CDR1	KSSKSLHLSNGNTYLN	159
	CDR2	WMSNLAS	160
	CDR3	MQHIEYPPT	161
GC194 (L) 1	CDR1	RSSKSLHSDITYLY	162
	CDR2	QMSNLAS	147
	CDR3	AQNLELPPT	163
GC194 (L) 2	CDR1	SASSSVSYMY	164
	CDR2	DTSNLAS	165
	CDR3	QQWSSYPLT	166
GC199 (L)	CDR1	KSSQSLHSDGKTFLN	167
	CDR2	LVSRLDS	168
	CDR3	CQGFHFPR	169
GC202 (L)	CDR1	RSSQSIHLSNGNTYLE	170
	CDR2	KVSNRFS	144
	CDR3	FQGSHPWT	171



## Example 19

### Measurement of ADCC activity using mouse bone marrow derived effector cell

#### 19.1 Preparation of mouse bone marrow derived effector cell solution

Bone marrow cells were collected from the femur of an SCIDmouse (CLEA Japan, Inc., male, 10 weeks old), and suspended in 10% FBS/RPMI 1640 medium at  $5 \times 10^5$  cells/mL. Mouse GM-CSF (PeproTech) and human IL-2 (PeproTech) were added at 10 ng/mL and 50 ng/mL, respectively, and the cells were cultured in a 5% carbon dioxide gas incubator at 37°C for 5 days. After the cultivation, the cells were scraped off with a scraper and washed with the medium once. Then, the cells were suspended in 10% FBS/RPMI 1640 medium at  $5 \times 10^6$  cells/mL, and used as a mouse bone marrow derived effector cell solution.

#### 19.2 Preparation of target cell

A human hepatoma cell line, HuH-7, was maintained and subcultured with DMEM medium (SIGMA) containing 10% FBS (ThermoTrace). The cells were dissociated from the dish using Cell Dissociation Buffer (Invitrogen), dispensed to each well of a 96-well U-bottomed plate (Falcon) at  $1 \times 10^4$  cells/well, and cultured for 1 day. After the cultivation, 5.55 MBq of chromium-51 was added, and the cells were cultured in a 5% carbon dioxide gas incubator at 37°C for 1 hour. These cells

were washed with the medium once, and 50  $\mu$ L of 10% FBS/RPMI 1640 medium was added and used as a target cell.

### 19.3 Chromium release test (ADCC activity)

To the target cell, 50  $\mu$ L of an antibody solution prepared at different concentrations was added, and reacted on ice for 15 minutes. Subsequently, 100  $\mu$ L of the mouse bone marrow derived effector cell solution ( $5 \times 10^5$  cells/well) was added, and cells were cultured in a 5% carbon dioxide gas incubator at 37°C for 4 hours. After the cultivation, the plate was centrifuged, and the radioactivity in 100  $\mu$ L of the culture supernatant was measured using a gamma counter. The specific chromium release rate was obtained by the following formula.

$$\text{Specific chromium release rate (\%)} = (A-C) \times 100 / (B-C)$$

"A" represents the mean value of the radioactivities (cpm) in each well, "B" represents the mean value of the radioactivities (cpm) in the wells in which 100  $\mu$ L of 2% NP-40 aqueous solution (Nonidet P-40, Code No. 252-23, Nacalai Tesque) and 50  $\mu$ L of 10% FBS/RPMI medium were added to the target cell, and "C" represents the mean value of the radioactivities (cpm) in the wells in which 150  $\mu$ L of 10% FBS/RPMI medium was added to the target cell. The test was carried out in triplicate and the mean value and the standard deviation were calculated for ADCC activity (%).

The results are shown in Fig. 9. It was revealed that

GC33 antibody shows an ADCC activity when the concentration of antibody is 0.1 µg/mL or higher, and shows stronger activity than GC199 antibody.

## Example 20

### Antitumor activity of GC33 antibody to mouse model transplanted with human hepatoma

#### 20.1 Preparation of mouse model transplanted with human hepatoma

A human hepatoma cell line, HuH-7, was prepared at  $5 \times 10^7$  cells/mL in a solution containing DMEM medium and MATRIGEL (BD Bioscience) at a ratio of 1:1. On the previous day, 100 µL of an anti-asialo GM1 antibody solution (Wako Pure Chemicals) was intraperitoneally administered to a SCID mouse (male, 5 weeks old, CLEA Japan, Inc.). The mouse was transplanted with 100 µL of the above-mentioned cell suspension ( $5 \times 10^6$  cells/mouse) subcutaneously in the abdominal area.

#### 20.2 Preparation and administration of antibody

Starting from the day 20 after the cell transplantation, an antibody solution prepared on the day of administration at 0.5 mg/mL (group of administration of 5 mg/kg) and at 0.1 mg/mL (group of administration of 1 mg/kg) with PBS(-) was administered to the mouse model transplanted with a human hepatoma cells at 10 mL/kg via the tail vein once a week for

3 weeks. As a negative control, PBS(-) (vehicle) was administered at 10 mL/kg via the tail vein once a week for 3 weeks in a similar manner. Both groups consisted of 6 mice each.

### 20.3 Evaluation of antitumor effect

The antitumor effect of GC33 antibody on the mouse model transplanted with human hepatoma cells was evaluated with the change in tumor volume with time and tumor weight at 1 week after the final administration. The tumor volume was calculated by the following formula.

Tumor volume = (major axis) x (minor axis) x (minor axis)/2

As shown in Fig. 10, a significant inhibition of tumor growth was observed in the GC33 antibody group compared with the vehicle group.

Consequently, GC33 was shown to have an antitumor effect on the mouse model transplanted with a human hepatoma cells.

#### Example 21

#### Preparation of GC33 mouse-human chimeric antibody

The H chain and the L chain of GC33 were amplified by PCR using a synthetic oligonucleotide, which is complementary to the 5'-terminal nucleotide sequences and has a Kozak sequence and a HindIII site, and a synthetic oligonucleotide, which is complementary to the 3'-terminal nucleotide sequences

and has a BamHI site. After digestion with HindIII and BamHI, the obtained PCR product was cloned into an expression vector, HEFg $\gamma$ 1, in which a human IgG1 constant region was inserted, and an expression vector, HEFg $\kappa$ , in which a human kappa chain constant region was inserted (Sato et al., Mol Immunol. 1994; 371-381). The vectors were introduced into a CHO cell (DG44 cell line) in accordance with the above-mentioned method, and a stably expressing cell line was established. The antibody was purified from the culture supernatant using Hi Trap ProteinG HP (Amersham). The concentration of IgG in the culture supernatant was measured by a human IgG sandwich ELISA using goat anti-human IgG (BIOSOURCE) and goat anti-human IgG alkaline phosphatase conjugate (BIOSOURCE), and the concentration was determined by the comparison with a commercially available human IgG (Cappel).

#### Example 22

##### Measurement of CDC activity and ADCC activity using GC33 mouse-human chimeric antibody

In accordance with the methods described in Examples 16 and 17, the CDC activities and ADCC activities of GC33, M3C11 and M1E7 mouse-human chimeric antibodies were measured. As for the target cell, the CHO cell expressing full-length GPC3 was used for measuring the CDC activity and HepG2 was used for measuring the ADCC activity. The results are shown in Fig.

11 and Fig. 12, respectively. It was revealed that, in either test system, GC33 shows a strong CDC activity and ADCC activity compared with the other two antibodies.

#### Example 23

##### Epitope analysis for GC33

To determine the epitope for GC33 in detail, fusion proteins of a further shorter C-terminal peptide of GPC3 and GST were prepared, and analyzed by Western blotting. The prepared GPC3-derived peptide sequences contained in the GST-fusion protein are shown in Fig. 13. Since GC33 can bind to GC-4 (aa 537-563), but cannot bind to GC-5 (aa 550-563), it was considered that the epitope is located in a region containing at least part of the aa 537-550 region. First, the peptides GC-6 (G N S Q Q A T P K D N E I S (SEQ ID NO: 93)), GC-7 (G N S Q Q A T P (SEQ ID NO: 94)), GC-8 (Q Q A T P K D N (SEQ ID NO: 95)) and GC-9 (T P K D N E I S (SEQ ID NO: 96)) were prepared. A forward oligo DNA and a reverse oligo DNA were prepared which were designed in such a manner that the cleavage site of EcoRI recognition sequence is attached to the 5' end and the cleavage site of SalI recognition sequence is attached to the 3' end, respectively. The synthesis of the oligo DNAs was done by Espec Oligo Service. The DNA was purified with C-18 cartridge, phosphorylated at the 5' end and used for the analysis. Twenty-five microliters of the forward



oligo DNA (10  $\mu$ M) and 25  $\mu$ L of the reverse oligo DNA (10  $\mu$ M) were mixed and reacted at 94°C for 5 minutes, at 37°C for 10 minutes, and at room temperature for 15 minutes, then left at 4°C for 10 minutes to anneal the forward oligo DNA and the reverse oligo DNA. The concentration of the oligos was determined by the absorbance measurement at the molar ratio of the insert to the vector of 3:1. The oligos were cloned into EcoRI- and SalI-digested pGEX4T-3, and the nucleotide sequence was confirmed. A GST-fusion protein was prepared in accordance with the above-mentioned method, and purified using Gluthatione Sepharose 4B. The purified proteins were separated by SDS-PAGE under reducing conditions, and analyzed by Western blotting using GC33. As a result, the antibody GC33 could not detect any GST-fusion protein strongly, suggesting that a longer sequence at the C-terminal side is needed for the binding of GC33 (Fig. 14). Based on the above prediction, GC-11 (A T P K D N E I S T (SEQ ID NO: 97)), GC-12 (P K D N E I S T F H (SEQ ID NO: 98)), GC-13 (D N E I S T F H N L (SEQ ID NO: 99)) and GC-14 (E I S T F H N L G N (SEQ ID NO: 100)) were prepared and evaluated in the same manner. As a result, GC-11, GC-12 and GC-13 bound to GC33 more strongly, suggesting that the epitope for GC33 is located in the sequence from 544th to 553rd (P K D N E I S T F H) at the C-terminus of GPC3.

Example 24

### Humanization of GC33

Antibody sequence data were obtained from publicly disclosed Kabat Database (<ftp://ftp.ebi.ac.uk/pub/databases/kabat/>) and from ImMunoGeneTics Database (IMGT). The H chain variable region and the L chain variable region were separately subjected to homology search. As a result, the H chain variable region was found to have a high homology with DN13 (Smithson et al., Mol Immunol. 1999; 36: 113-124), and the L chain variable region was found to have a high homology with homo sapiens IGK mRNA for immunoglobulin kappa light chain VLJ region, partial cds, clone: K64 of the accession number of AB064105. The signal sequence of the accession number of S40357 that has a high homology with AB064105 was used as a signal sequence of the L chain. The complementarity determining region (hereinafter referred to as CDR) of GC33 were transplanted into the framework regions (hereinafter referred to as FR) of these human antibodies to prepare a humanized antibody.

Specifically, synthetic oligo DNAs of approximately 50 bases were designed in such a manner that approximately 20 bases of them were hybridized and these synthetic oligo DNAs were assembled together by the PCR method to prepare genes encoding each of the variable regions. They were digested at the HindIII site inserted in the end of the 5'-terminal synthetic oligo DNA and the BamHI site inserted in the end of the 3'-terminal

synthetic oligo DNA. The fragments were cloned into an expression vector, HEFgyl, in which a human IgG constant region was cloned, or an expression vector, HEFgk1, in which a human kappa chain constant region was cloned (Sato et. al., Mol Immunol. 1994; 371-381). The H chain and the L chain of the humanized GC33 constructed as above were named ver.a, respectively. The binding activity of the humanized GC33, whose H chain and the L chain were both ver.a (ver.a/ver.a), was lower than that of an antibody with mouse GC33 variable regions (mouse/mouse). Antibodies were constructed in which the mouse GC33 sequence and the ver.a sequence were chimerically combined (mouse/ver.a, ver.a/mouse) with regard to the H chain and the L chain, and their binding activities were evaluated. As a result, a decrease in binding activity was observed in ver.a/mouse, indicating that the decrease in binding activity due to amino acid replacement was attributed to the H chain (Fig. 15). Then, modified H chains, ver.c, ver.f, ver.h, ver.i, ver.j, ver.k were prepared. All these humanized GC33 showed a binding activity equivalent to that of a chimeric antibody having the mouse GC33 variable region (Fig. 15). The nucleotide sequences of the humanized GC33 H chain variable regions, ver.a, ver.c, ver.f, ver.h, ver.i, ver.j, ver.k were shown in SEQ ID NOs: 77, 78, 79, 80, 81, 82 and 83, respectively, and the amino acid sequences thereof were shown in SEQ ID NOs: 84, 85, 86, 87, 88, 89 and 90, respectively. The nucleotide

sequence and the amino acid sequence of a humanized GC33 L chain variable region, ver.a are shown in SEQ ID NOs: 91 and 92, respectively. In humanized GC33 H chain variable regions ver.i, ver.j and ver.k, the 6th glutamic acid residue is replaced with glutamine residue. The heat stability of these antibodies was significantly increased.

#### Example 25

##### Modification of humanized GC33 L chain

As for the deamidation of protein, the reaction rate constant of deamidation is known to be dependent on the primary sequence. It is also known that Asn-Gly is particularly susceptible to deamidation (Rocinson et. al., Proc. Natl. Acad. Sci. USA 2001; 98; 944-949). As for Asn33 in the CDR1 of a humanized GC33 L chain ver.a shown in SEQ ID NO: 91, the primary sequence is Asn-Gly, which is predicted to be very susceptible to deamidation.

To evaluate the effect of deamidation of Asn33 on the binding activity, a modified antibody was prepared in which Asn33 was replaced with Asp. A point mutation was introduced using a Quick Change Site-Directed Mutagenesis Kit (Stratagene) was used. More specifically, 50  $\mu$ L of a reaction mixture containing 125 ng of a sense primer (CTT GTA CAC AGT GAC GGA AAC ACC TAT: SEQ ID NO: 172), 125 ng of an antisense primer (ATA GGT GTT TCC GTC ACT GTG TAC AAG: SEQ ID NO: 173),

5  $\mu$ L of 10x reaction buffer, 1  $\mu$ L of dNTP mix, 10 ng of HEFgk into which a humanized GC33 L chain ver.a had been cloned and 1  $\mu$ L of Pfu Turbo DNA Polymerase was subjected to PCR of 12 cycles consisting of 95°C for 30 seconds, 55°C for 1 minute and 68°C for 9 minutes. Subsequently, a restriction enzyme, DpnI, was added and digestion was carried out at 37°C for 2 hours, and the digested product was introduced into XL1-Blue competent cell attached to the kit, whereby a transformant was obtained. The variable region was cleaved out from the clone in which each mutation was properly introduced, and cloned into HEFgk again. It was introduced into a COS7 cell using Fugene 6 (Roche) together with HEFgy1, in which a humanized GC33 H chain ver.k had been cloned. The antibody transiently expressed in the cell was recovered from the culture supernatant. The concentration of antibody was determined by a sandwich ELISA using the anti-human IgG antibody. The binding activity of the modified antibody was evaluated by an ELISA using an immunoplate coated with the soluble form of GPC3 core protein. As shown in Fig. 18, the binding activity was lost in the modified antibody (N33D) in which Asn33 had been replaced with Asp, suggesting that the effect of the deamidation of Asn33 on the binding activity was significant.

As a method of suppressing deamidation of Asn33, replacement of Gly34 with another amino acid has been reported (International Patent Application WO 03057881A1). In

accordance with the above-mentioned method, G34 was replaced with any of 17 amino acids other than Cys and Met using a Quick Change Site-Directed Mutagenesis Kit to prepare a series of modified antibodies, namely, G34A, G34D, G34E, G34F, G34H, G34N, G34P, G34Q, G34I, G34K, G34L, G34V, G34W, G34Y, G34R, G34S and G34T. These antibodies were transiently expressed in COS7 cells, and the binding activity was evaluated using the culture supernatant. It was found that the binding activity is maintained even if G34 is replaced with another amino acid, except for Pro (G34P) and Val (G34V).

The amino acid sequences of the light chain CDR1 of the above-mentioned modified antibodies are shown in SEQ ID NO: 174 (G34A), SEQ ID NO: 175 (G34D), SEQ ID NO: 176 (G34E), SEQ ID NO: 177 (G34F), SEQ ID NO: 178 (G34H), SEQ ID NO: 179 (G34N), SEQ ID NO: 180 (G34T), SEQ ID NO: 181 (G34Q), SEQ ID NO: 182 (G34I), SEQ ID NO: 183 (G34K), SEQ ID NO: 184 (G34L), SEQ ID NO: 185 (G34S), SEQ ID NO: 186 (G34W), SEQ ID NO: 187 (G34Y), SEQ ID NO: 188 (G34R), SEQ ID NO: 189 (G34V) and SEQ ID NO: 190 (G34P), respectively. The amino acid sequences of the light chain variable regions of the above-mentioned modified antibodies are shown in SEQ ID NO: 191 (G34A), SEQ ID NO: 192 (G34D), SEQ ID NO: 193 (G34E), SEQ ID NO: 194 (G34F), SEQ ID NO: 195 (G34H), SEQ ID NO: 196 (G34N), SEQ ID NO: 197 (G34T), SEQ ID NO: 198 (G34Q), SEQ ID NO: 199 (G34I), SEQ ID NO: 200 (G34K), SEQ ID NO: 201 (G34L), SEQ ID NO: 202 (G34S), SEQ ID

NO: 203 (G34W), SEQ ID NO: 204 (G34Y), SEQ ID NO: 205 (G34R),  
SEQ ID NO: 206 (G34V) and SEQ ID NO: 207 (G34P), respectively.

#### Industrial Applicability

The antibody of the present invention can be used as a cell growth inhibitor, an anticancer agent and an agent for diagnosis of cancers.

#### Brief Description of the Drawings

Fig. 1 shows the binding activity of the anti-GPC3 antibody to a CHO cell, a CHO cell expressing full-length GPC3, HepG2 and HuH-7, which was evaluated by flow cytometry. M1E7 (solid line) and M11F1 (dashed line) were used at a concentration of 5 µg/mL, respectively.

Fig. 2 is a table showing the results of epitope classification by a competitive ELISA. The degrees of competitive inhibition against the binding of the biotinylated anti-GPC3 antibody are indicated by percentage. The epitopes were classified into 5 groups, a to e, according to the competitive inhibition pattern.

Fig. 3 shows the results of evaluating by Western blotting whether an anti-GPC3 antibody binds to the N-terminal fragment of 40 kDa of the soluble form of GPC3 core protein or to the C-terminal fragment of 30 kDa thereof. It was found that L9G11 binds to the N-terminal fragment and M3C11 binds

to the C-terminal fragment.

Fig. 4 shows the results of detecting a secreted form of GPC3 is present in the culture supernatant of HepG2 by a sandwich ELISA. It was strongly detected with the combination of antibodies that bind to the N-terminal fragment such as M6B1, M18D4 or M19B11, and it was not strongly detected with an antibody that binds to the C-terminal fragment such as M3C11, M13B3 or M3B8.

Fig. 5 shows the results of immunoprecipitation of the culture supernatant of HepG2 with the use of an anti-GPC3 antibody and detection of a secreted form of GPC3. The medium as a control (lanes 1 and 3) and the culture supernatant of HepG2 (lanes 2 and 4) were immunoprecipitated using M1E7 (lanes 1 and 2) and M10D2 (lanes 3 and 4). Secretory GPC3 was detected by M10D2 that binds to the N-terminal fragment.

Fig. 6 shows the results of analyzing the epitope of the antibodies that bind to the C-terminal fragment of GPC3 by Western blotting with the use of a fusion protein of the C-terminal peptide of GPC3 and GST. The soluble form of GPC3 core protein (lane 1), GST (lane 2), GC-1 (lane 3), GC-2 (lane 4), GC-3 (lane 5), GC-4 (lane 6) and GC-5 (lane 7) were subjected to SDS electrophoresis under reducing conditions, and detected by Western blotting using M3C11 and M11F1.

Fig. 7 shows the results of evaluating the CDC activity of the anti-GPC3 mouse-human chimeric antibody to a CHO cell



that expresses GPC3.

Fig. 8 shows the results of evaluating the ADCC activity of the anti-GPC3 mouse-human chimeric antibody to a CHO cell that expresses GPC3 and HepG2.

Fig. 9 shows the results of evaluating the ADCC activity of GC33 to a human hepatoma cell line, HuH-7, using a mouse bone marrow-derived effector cell.

Fig. 10 shows the results of evaluating the antitumor activity of GC33 antibody to a mouse model transplanted with human hepatoma.

Fig. 11 shows the results of evaluating the CDC activity of the mouse-human chimeric antibody GC33 to a CHO cell that expresses GPC3.

Fig. 12 shows the results of evaluating the ADCC activity of the mouse-human chimeric antibody GC33 to HepG2.

Fig. 13 shows GPC3-derived sequences contained in GST-fusion proteins (GC-6, 7, 8, 9, 10, 11, 12, 13 and 14) prepared for analyzing the epitope of GC33.

Fig. 14 shows the results of Western blotting with the use of GC33 after separating GST, GC-7, 8, 9, 10, 11, 12, 13 and 14 by SDS-PAGE under reducing conditions.

Fig. 15 shows the results of evaluating the binding activity of humanized GC33 to GPC3 by an ELISA.

Fig. 16 shows an antibody panel which summarizes isotypes and the results of an ELISA, BIAcore, FACS, an epitope analysis

and an immunoprecipitation test for clones derived from a mouse immunized with a soluble form of GPC3.

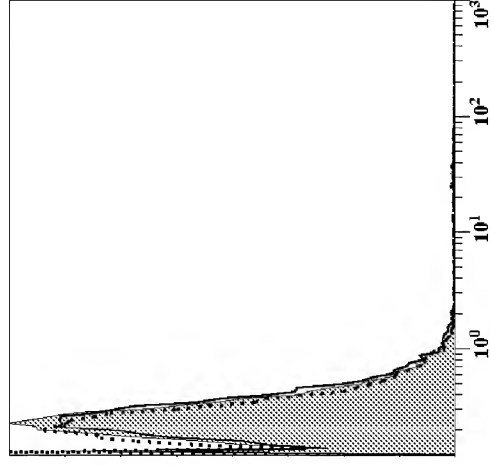
Fig. 17 shows an antibody panel in which isotypes and the results of an ELISA, FACS and an epitope analysis for clones derived from a mouse immunized with GC-3 are summarized.

Fig. 18 shows the results of evaluating the binding activity of the modified antibodies to the soluble form of GPC3 core protein by an ELISA. Gly34 located at CDR1 in a humanized GC33 L chain variable region was replaced with any of 17 amino acids other than Cys and Met.

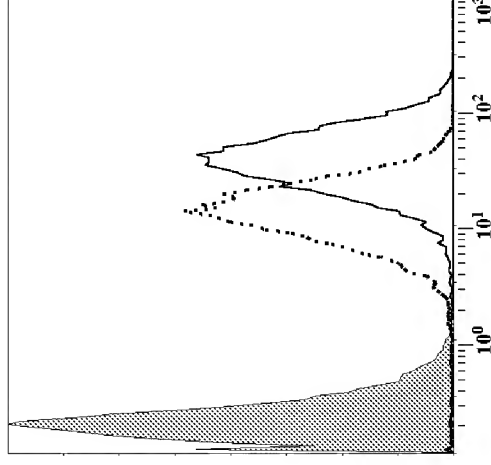
## ABSTRACT

An antibody capable of binding to a specific region of glypican 3, as well as a humanized antibody created based on that antibody are disclosed. The anti-GPC3 antibody of the invention has a higher ADCC activity and CDC activity compared with those of a conventional antibody. The antibody of the present invention is useful as a cell growth inhibitor, an anticancer agent and an agent for diagnosis of cancers.

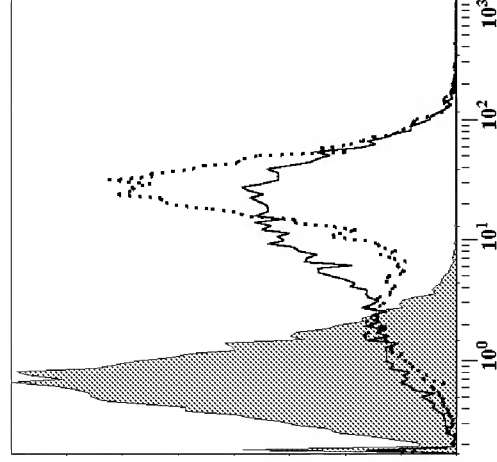
CHO



CHO/human GPC3



HepG2



HuH-7

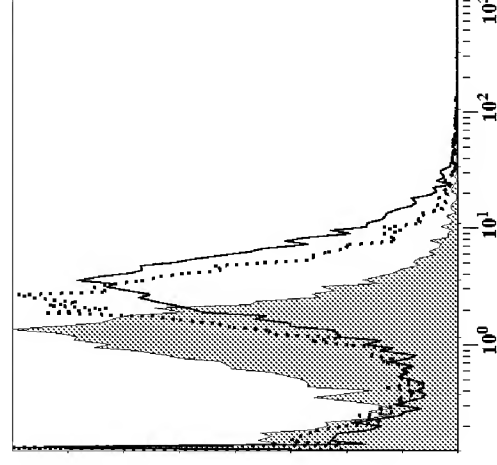


FIG.1

Mab	Inhibition of the binding of indicated biotin-Mab to sGPC3 core (%)								Topographical epitope
	M3C11	M1E7	M11F1	M6B1	M18D4	M5B9	M10D2	L9G11	
M3C11	<u>96.8</u>	<u>96.2</u>	12.0	11.2	10.3	9.1	39.9	33.1	a
M13B3	<u>71.3</u>	<u>95.7</u>	15.6	9.3	2.7	-1.8	22.3	24.9	
M1E7	26.0	<u>83.9</u>	14.3	7.7	9.0	6.4	14.8	38.0	
M3B8	1.8	28.4	<u>97.4</u>	15.2	12.6	3.6	29.0	36.0	b
M11F1	2.0	3.9	<u>81.8</u>	5.6	-1.5	5.4	24.6	20.9	
M19B11	-4.1	-9.4	3.1	<u>87.6</u>	44.1	31.0	14.2	-9.8	c
M6B1	-5.7	-2.7	13.9	<u>85.6</u>	<u>51.9</u>	44.4	6.7	1.1	
M18D4	0.2	0.5	-0.3	38.6	<u>89.0</u>	<u>89.4</u>	19.1	6.7	
M5B9	0.1	2.7	5.3	23.2	<u>77.3</u>	<u>77.3</u>	13.4	10.3	
M10D2	-7.7	0.6	7.8	-5.9	-21.6	-10.3	<u>79.2</u>	7.2	d
L9G11	2.1	-4.8	6.0	10.1	15.7	6.4	1.2	<u>92.2</u>	e

FIG.2

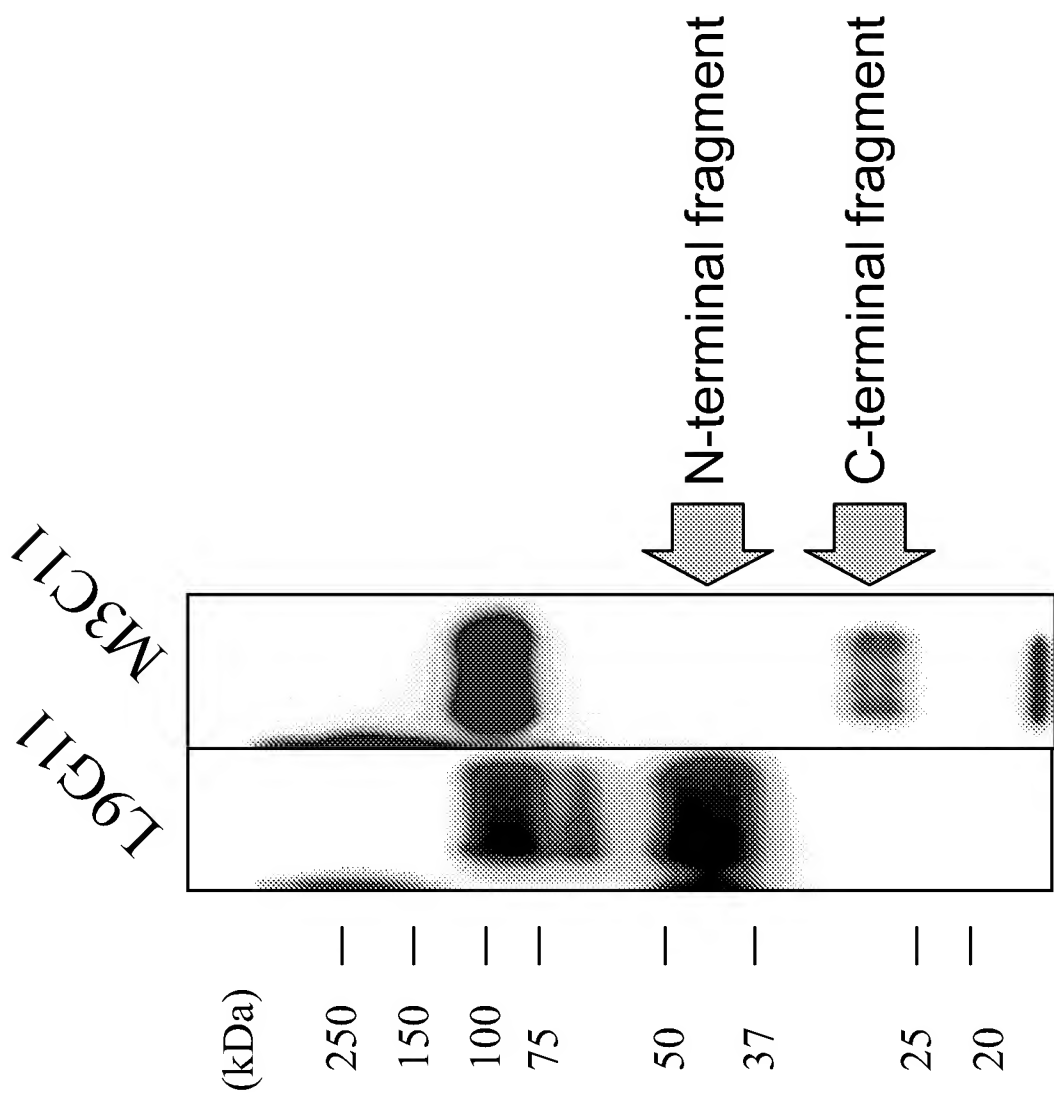


FIG.3

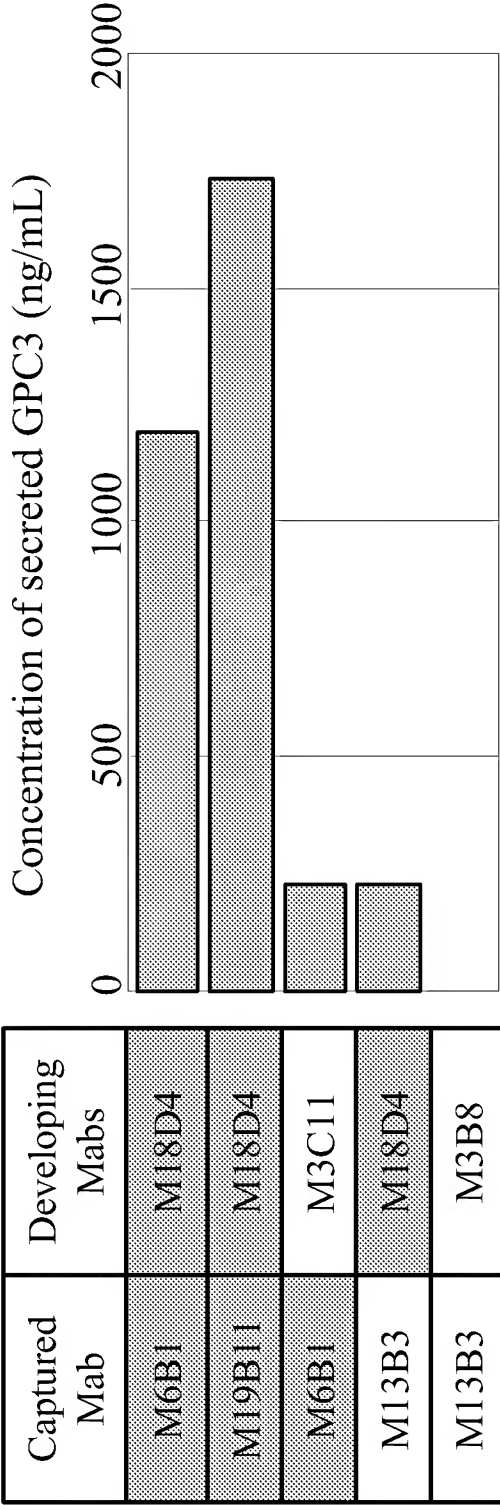


FIG.4

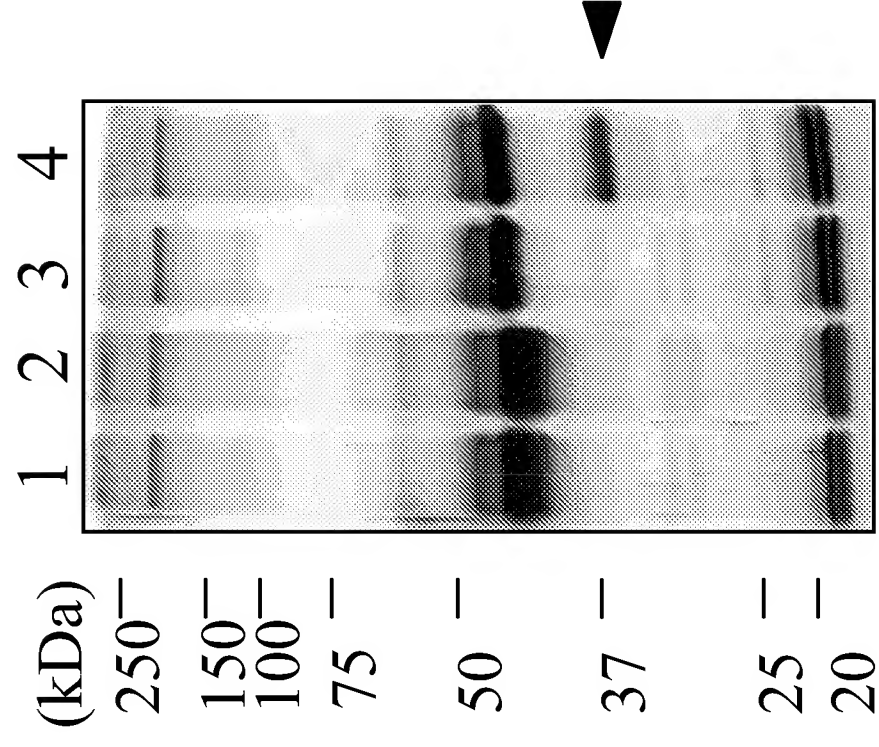


FIG.5



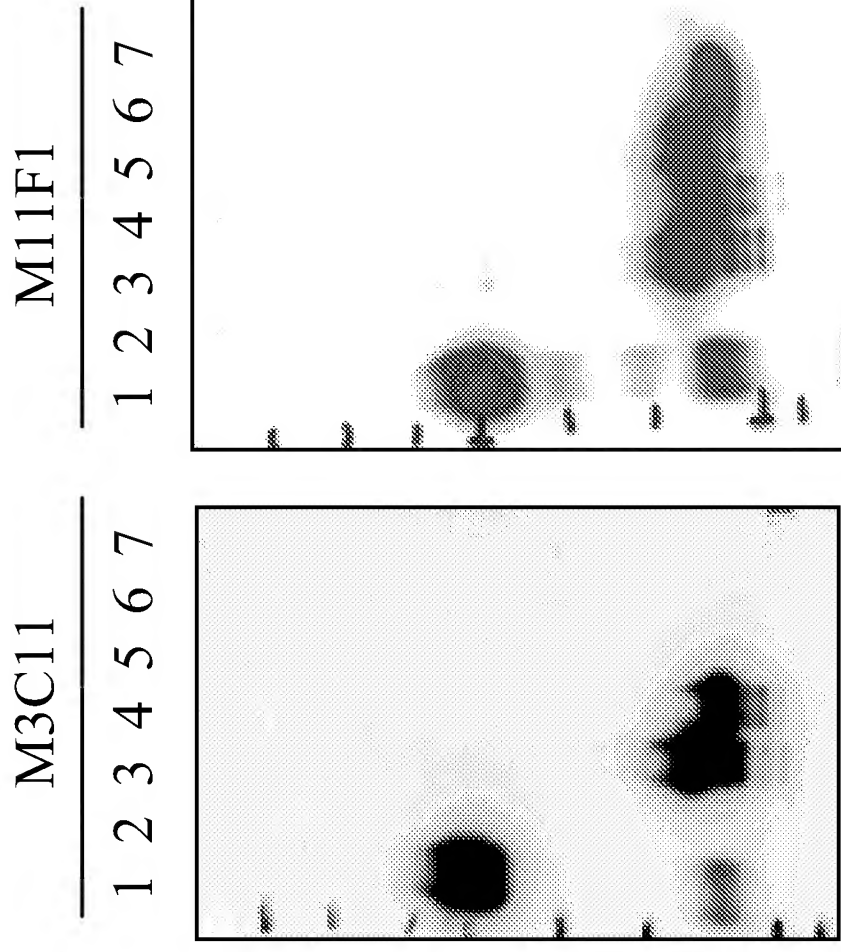


FIG.6

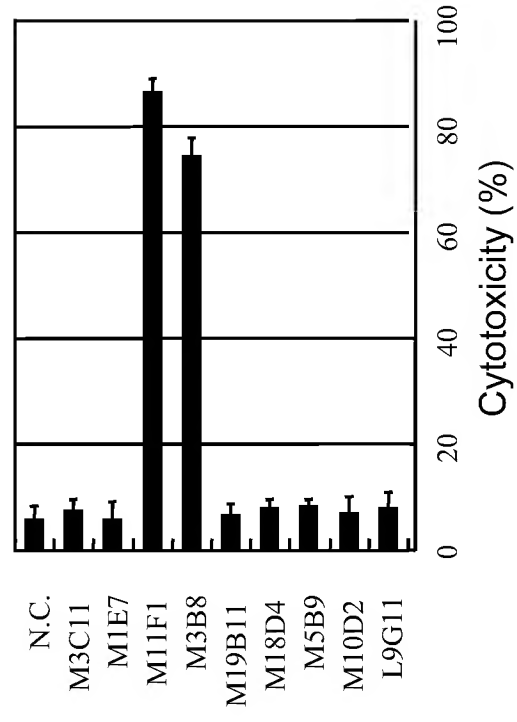


FIG.7

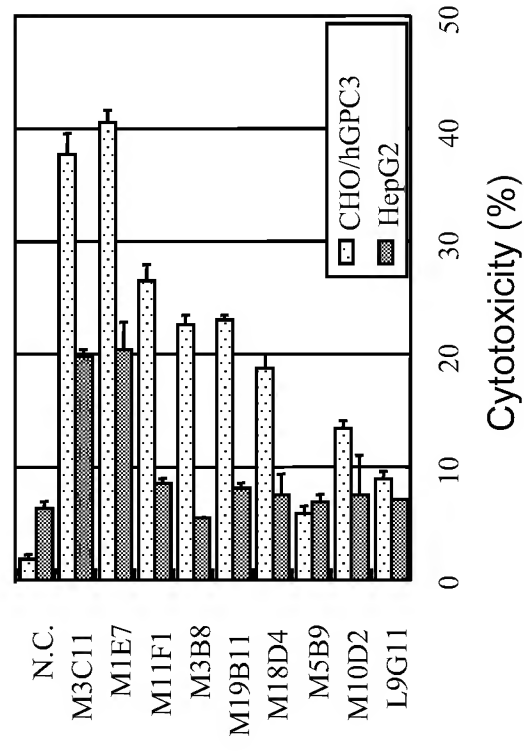


FIG.8

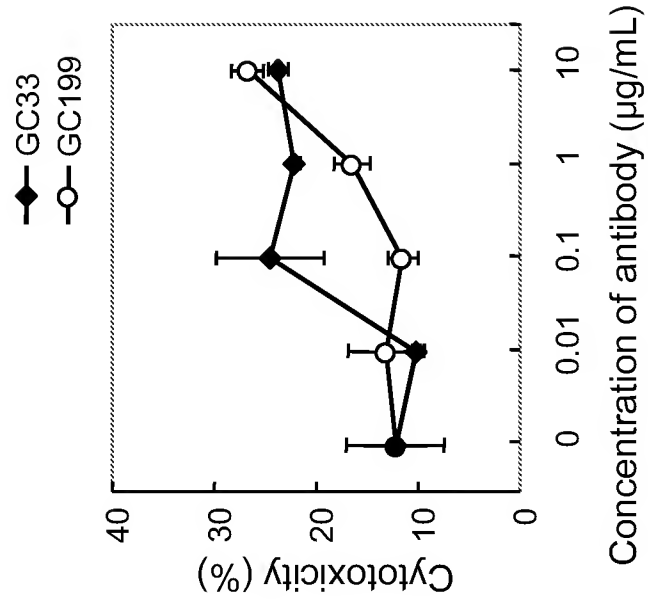


FIG.9

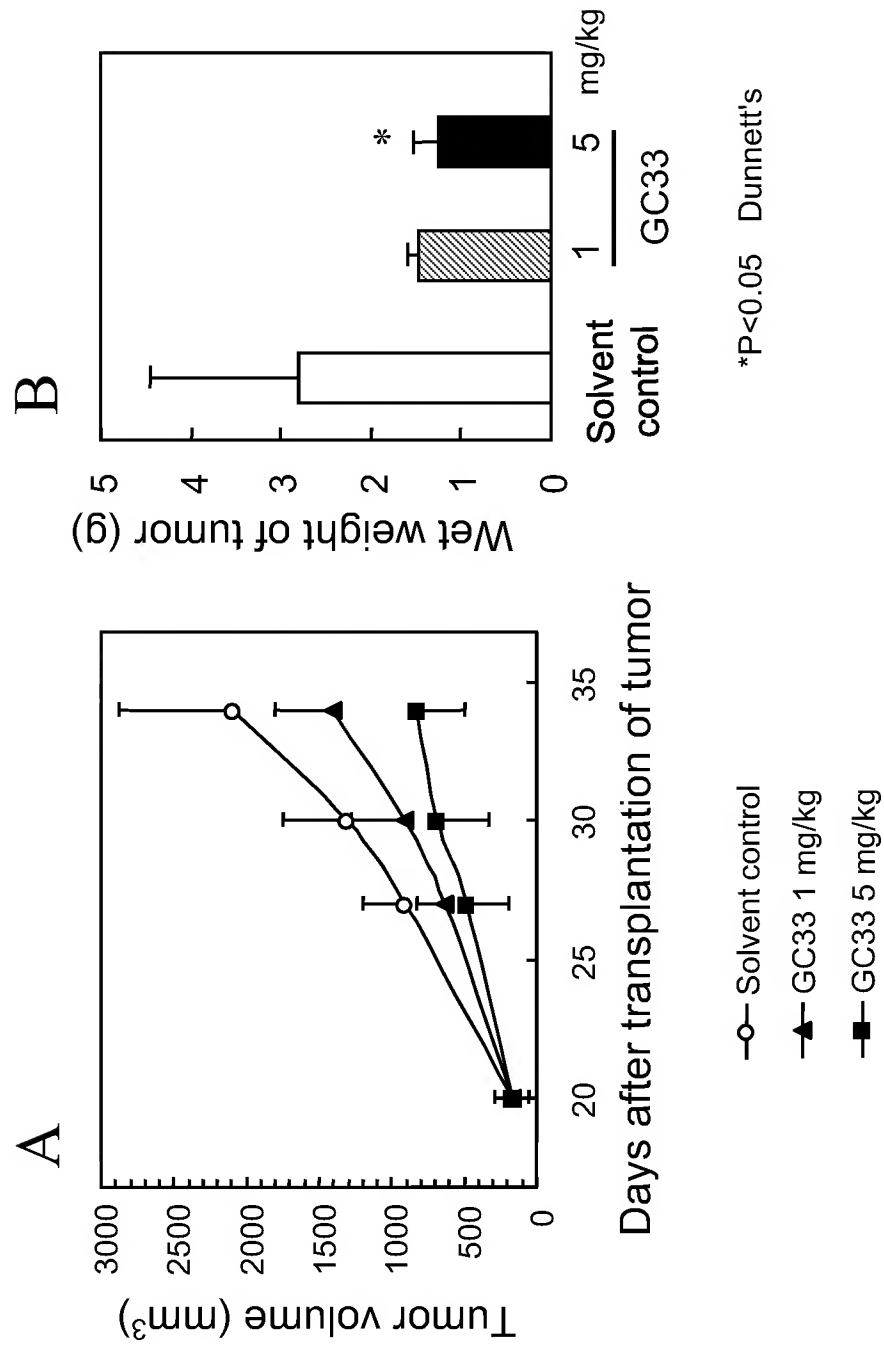


FIG.10

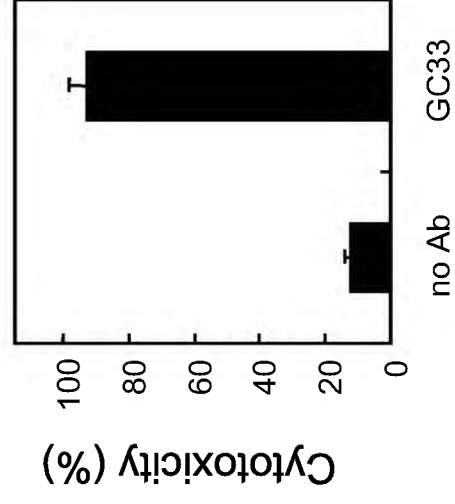
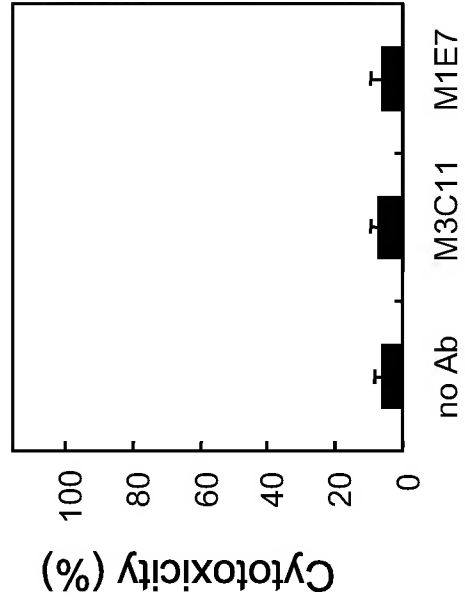


FIG.11

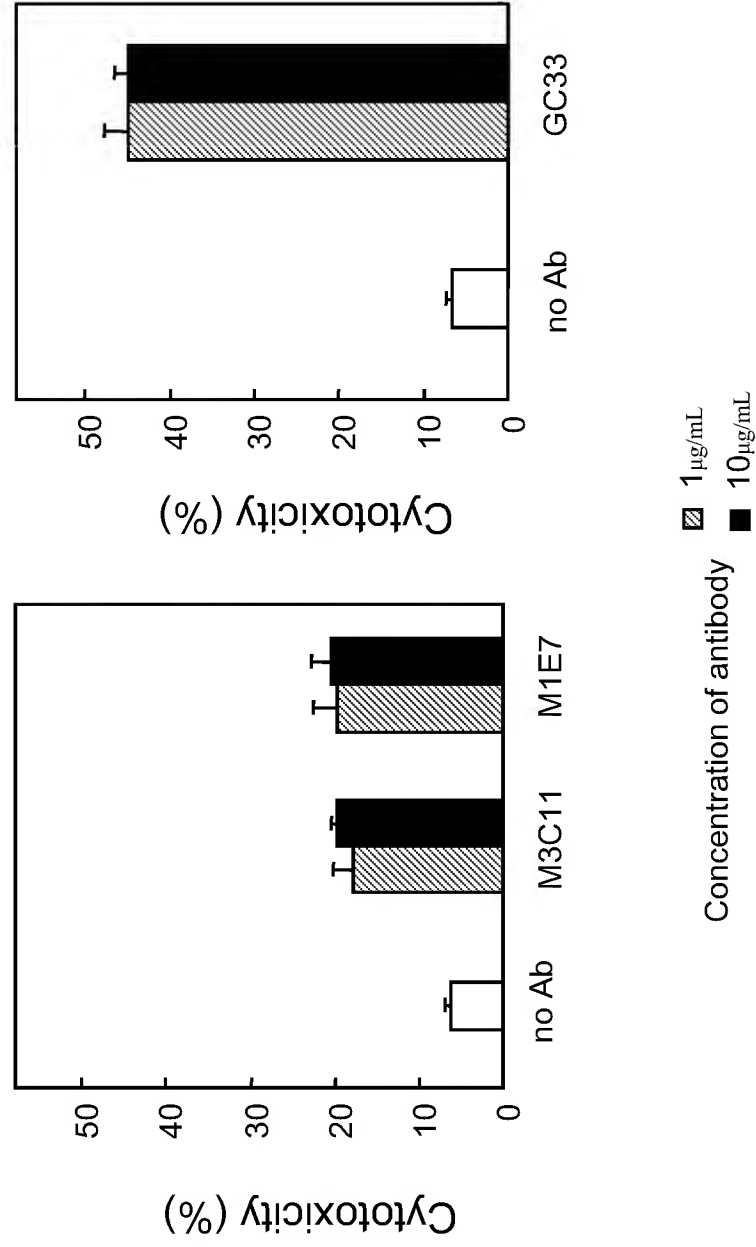


FIG.12

	GC33 WB
GC-4	GNSQQATPKDNEISTEHNLGNVHSPILK
GC-5	STEHNLGNVHSPILK
GC-6	GNSQQATPKDNEIS
GC-7	GNSQQATP
GC-8	QQATPKDN
GC-9	TPKDNELIS
GC-11	ATPKDNELIST
GC-12	PKDNELISTEH
GC-13	DNELISTEHNI
GC-14	ELSTEHNLGN

FIG.13



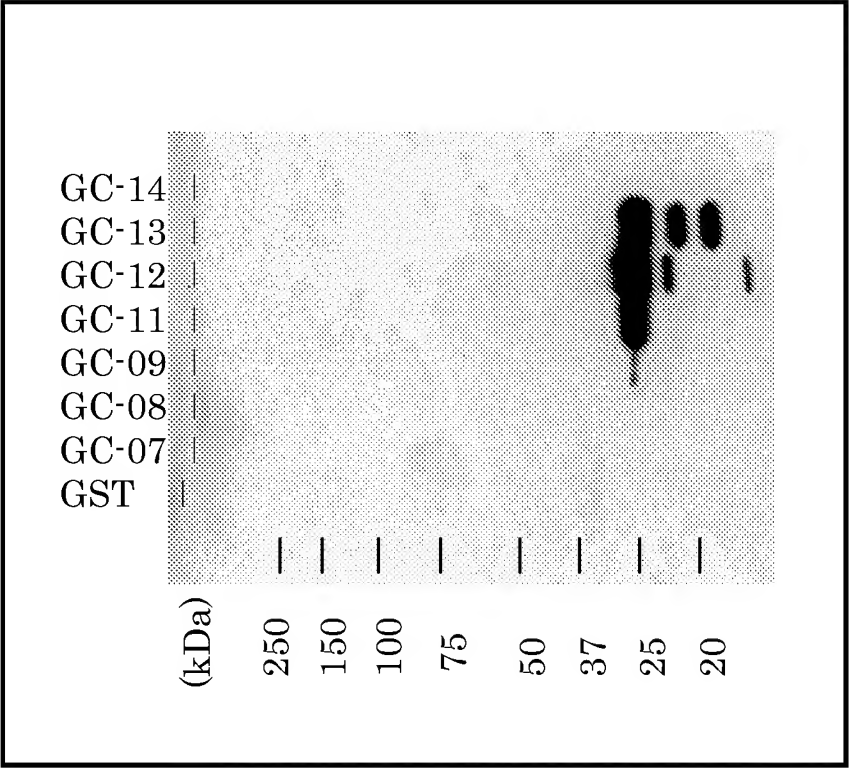


FIG.14

Clone ID	Isotype	ELISA		BIACORE			EPI TOPE		FACS				Immuno-precipitation
		EC50 (nM)		ka (1/Ms /10 <sup>5</sup> )	kd (1/s x10 <sup>5</sup> )	KD (nM)	Competitive ELISA	western blotting	CHO/ human GPC3	HepG2	HuH-7	CHO	
M3C11	IgG1	0.12		2.5	6.5	0.3	a	GC-2	83	59	9	-	△
M13B3	IgG1	0.25		N.D.	N.D.	N.D.			N.D.	N.D.	-	N.D.	
M1E7	IgG1	0.96		1.5	42.7	2.8			28	16	5	-	-
M3B8	IgG1	0.56		2.7	467.0	17.6	b	GC-4	12	6	3	-	×
M11F1	IgG2b	2.17		1.4	3.5	0.2			9	4	2	-	-
L9G11	IgG1	0.62		1.3	140.0	10.7	c	N	29	26	4	-	○
M19B11	IgG1	0.18		1.5	17.1	1.1	c		39	44	3	-	○
M6B1	IgG1	5.51		N.D.	N.D.	N.D.			37	34	3	-	○
M18D4	IgG1	0.85		0.8	49.6	6.2			22	20	2	-	○
M5B9	IgG1	1.13		0.6	139.0	23.5			25	23	2	-	○
M10D2	IgG1	1.04		1.1	49.6	4.7			20	29	3	-	○

FIG.16

Clone ID	Isotype	ELISA	EPITOPE		FACS			
		EC50 (nM)	Competitive ELISA	Western blotting	CHO/ human GPC3	HepG2	HuH-7	CHO
GC199	IgG1	0.10	b	GC-4	15.5	12.2	2.4	-
GC202	IgG1	0.10			6.0	0.9	1.4	-
GC33	IgG2a	0.24			82.7	52.0	8.4	-
GC179	IgG2b	5.61	f	GC-3	7.1	6.5	3.8	-
GC194	IgG1	3.83			5.0	7.9	1.5	-

FIG.17

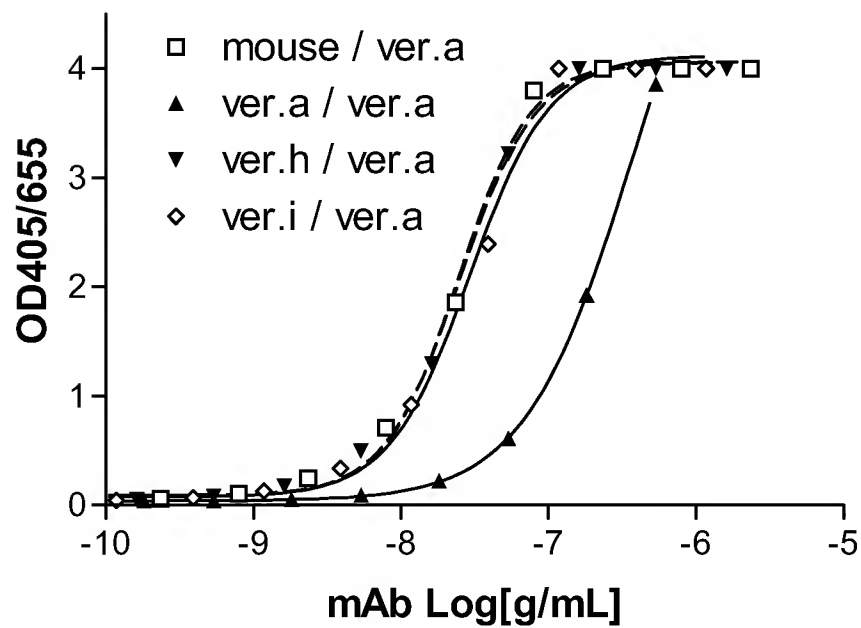
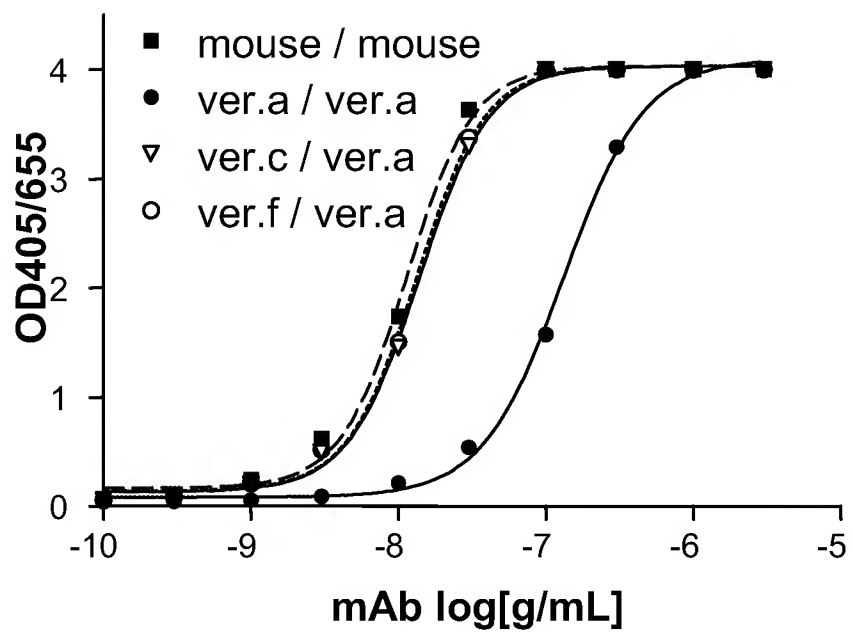


FIG.15

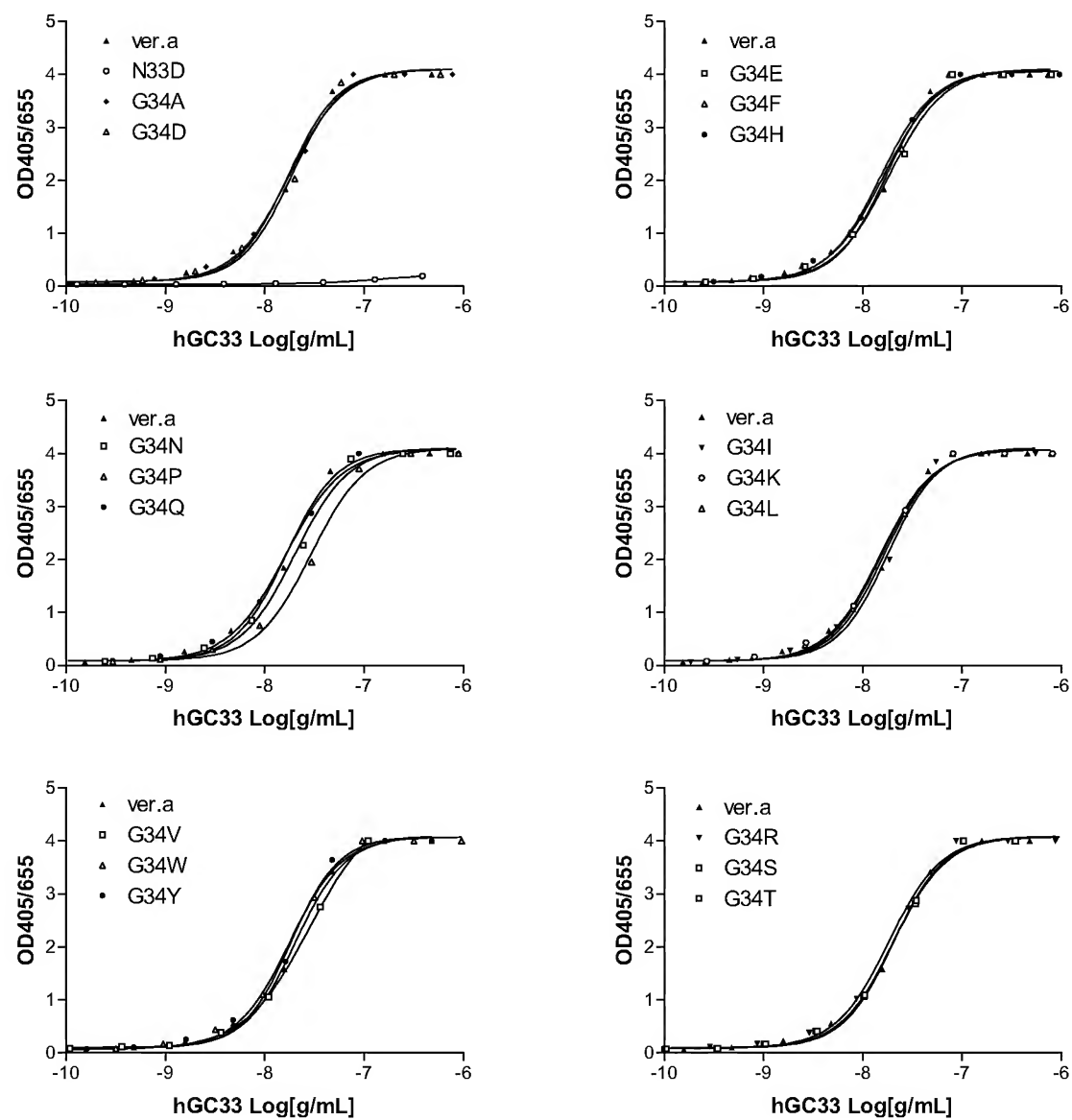


FIG.18

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gagtacaagt	gcaagggtctc	caacaaagcc	ctcccagccc	ccatcgagaa	aaccatctcc	1080
aaagccaaag	ggcagccccg	agaaccacag	gtgtacaccc	tgcccccatc	ccgggatgag	1140
ctgaccaaga	accagggtcag	cctgacctgc	ctgggtcaaag	gcttctatcc	cagcgacatc	1200
gccgtggagt	gggagagcaa	tgggcagccg	gagaacaact	acaagaccac	gcctcccgtg	1260
ctggactccg	acggctcctt	cttcctctac	agcaagctca	ccgtggacaa	gagcaggtgg	1320
cagcagggga	acgtcttctc	atgctccgtg	atgcatgagg	ctctgcacaa	ccactacacg	1380
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<210> 14

<211> 354

<212> DNA

<213> Mus musculus

<400> 14

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cagccttcag	ggatgggtct	ggagtggctg	gccaaacattt	ggtgggtatga	tgctaagtac	180
tataactctg	acctgaagag	ccggctcaca	atctccaagg	atacctccaa	caaccaggtg	240
ttcctcaaga	tctccagtgt	ggacacttca	gatactgcc	catactactg	tgtctcaaatg	300
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<210> 15

<211> 354

<212> DNA

<213> Mus musculus

<400> 15

caggtcactc	tgaaagagtc	tggccctggg	atattgcagc	cctcccagac	cctcagttctg	60
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acttggttctt	tctctgggtt	ttcactgagc	atztatggta	tgggtgtagg	ttggattcgt	120
cagccttcag	ggaagggctt	ggagtggctg	gccaacattt	ggtggaatga	tgataagtac	180
tataactcag	ccctgaagag	ccggctcaca	atctccaagg	atacctccaa	caaccaggta	240
ttcctcaaga	tctccagtgt	ggacactgca	gatactgcca	catactactg	tgctcaaata	300
ggttactttct	actttgacta	ctgggggcaa	ggcaccactc	tcacagtctc	ctca	354

<210> 16

<211> 1416

<212> DNA

<213> Mus musculus

<400> 16

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tgtgcagcct	ctggatccac	tttcagtaac	tatgccatgt	cttgggttcg	ccagactcca	180
gagaagaggc	tggagtgggt	cgcagccatt	gatagtaatg	gaggtaccac	ctactatcca	240
gacactatga	aggaccgatt	caccatttcc	agagacaatg	ccaagaacac	cctgtacctg	300
caaatgaaca	gtctgaggtc	tgaagacaca	gcctttttatc	actgtacaag	acataatgga	360
gggtatgaaa	actacggctg	gtttgcttac	tggggccaag	ggactctggg	cactgtctct	420
gcagctagca	ccaagggccc	atcggtcttc	cccctggcac	cctcctccaa	gagcacctct	480
gggggcacag	cggccctggg	ctgcctggtc	aaggactact	tccccgaacc	ggtgacgggtg	540
tcgtggaact	caggcgccct	gaccagcggc	gtgcacacct	tcccggctgt	cctacagtcc	600
tcaggactct	actccctcag	cagcgtgggtg	accgtgccct	ccagcagctt	gggcacccag	660
acctacatct	gcaacgtgaa	tcacaagccc	agcaacacca	aggtggacaa	gaaagttgag	720
cccaaactct	gtgacaaaac	tcacacatgc	ccaccgtgcc	cagcacctga	actcctgggg	780
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cctgaggtca	catgcgtggg	ggtggacgtg	agccacgaag	accctgaggt	caagttcaac	900
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aacagcacgt	accgtgtggg	cagcgtcctc	accgtcctgc	accaggactg	gctgaatggc	1020
aaggagtaca	agtgcaaggt	ctccaacaaa	gccctcccag	cccccatcga	gaaaaccatc	1080
tccaaagcca	aagggcagcc	ccgagaacca	caggtgtaca	ccctgcccc	atcccgggat	1140
gagctgacca	agaaccaggt	cagcctgacc	tgcttgggtc	aaggcttcta	tcccagcgac	1200
atcgccgtgg	agtgggagag	caatgggcag	ccggagaaca	actacaagac	cacgcctccc	1260
gtgctggact	ccgacggctc	cttcttcttc	tacagcaagc	tcaccgtgga	caagagcagg	1320
tggcagcagg	ggaacgtctt	ctcatgctcc	gtgatgcatg	aggctctgca	caaccactac	1380
acgcagaaga	gcctctccct	gtctccgggt	aaatga			1416

<210> 17

<211> 366

<212> DNA

<213> Mus musculus

<400> 17

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tctgtgcag	cctctggatt	cacttttcagt	agctatgcca	tgtcttgggt	tcgccagact	120
ccagagaaga	ggctggagtg	ggtcgcagcc	attaatatga	atggaggtac	cacctactat	180
ccagacacta	tgaaggaccg	attcaccatc	tccagagaca	atgccaaaga	caccctgtac	240
ctgcaaatga	gcagtctgag	gtctgaagac	tcagccttgt	attactgtac	aagacataat	300
ggaggggatg	aaaactacgg	ctggttttget	tactggggcc	aagggaactct	ggtcactgtc	360
tctgca						366

<210> 18

<211> 1413

<212> DNA

<213> Mus musculus

<400> 18

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gttcagctcc	agcagtctgg	gactgtgctg	gcaaggcctg	gggcttcagt	gaagatgtcc	120
tgcaaggcct	ctggctacac	ctttactggc	tactggatgc	gctgggtaaa	acagaggcct	180

ggacaggggtc	tggaatggat	tggcgctatt	tatcctggaa	atagtgatac	aacatacaac	240
cagaagttca	agggcaaggc	caaactgact	gcagtcacat	ctgtcagcac	tgcttacatg	300
gaactcagca	gcctgacaaa	tgaggactct	gcgggtctatt	actgttcaag	atcggggggac	360
ctaactgggg	ggtttgctta	ctgggggcaa	gggactctgg	tactgtctc	tacagccaaa	420
gctagcacca	agggcccatc	ggtcttcccc	ctggcaccct	cctccaagag	cacctctggg	480
ggcacagcgg	ccctgggctg	cctgggtcaag	gactacttcc	ccgaaccggg	gacgggtgtcg	540
tggaactcag	gcgccctgac	cagcggcgtg	cacaccttcc	cggctgtcct	acagtcctca	600
ggactctact	ccctcagcag	cgtgggtgacc	gtgccctcca	gcagcttggg	caccagacc	660
tacatctgca	acgtgaatca	caagcccagc	aacaccaagg	tggaacaaga	agttgagccc	720
aaatcttgtg	acaaaactca	cacatgccc	ccgtgccag	cacctgaact	cctggggggga	780
ccgtcagtct	tcctcttccc	cccaaaaccc	aaggacaccc	tcatgatctc	ccggaccct	840
gaggtcacat	gcgtgggtgt	ggacgtgagc	cacgaagacc	ctgaggtcaa	gttcaactgg	900
tacgtggacg	gcgtggagg	gcataatgcc	aagacaaagc	cgcgggagga	gcagtacaac	960
agcacgtacc	gtgtgggtcag	cgtcctcacc	gtcctgcacc	aggactgggt	gaatggcaag	1020
gagtacaagt	gcaagggtctc	caacaaagcc	ctcccagccc	ccatcgagaa	aaccatctcc	1080
aaagccaaag	ggcagccccg	agaaccacag	gtgtacaccc	tgcccccatc	ccgggatgag	1140
ctgaccaaga	accagggtcag	cctgacctgc	ctgggtcaaag	gcttctatcc	cagcgacatc	1200
gccgtggagt	gggagagcaa	tgggcagccg	gagaacaact	acaagaccac	gcctcccgtg	1260
ctggactccg	acggctcctt	cttcctctac	agcaagctca	ccgtggacaa	gagcagggtgg	1320
cagcagggga	acgtcttctc	atgctccgtg	atgcatgagg	ctctgcacaa	ccactacacg	1380
cagaagagcc	tctccctgtc	tccgggtaaa	tga			1413

<210> 19

<211> 357

<212> DNA

<213> Mus musculus

<400> 19

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tcttgcaagg	cttctggcta	cacctttacc	ggctactgga	tgcactgggt	aaaacagagg	120
cctggacagg	gtctggaatg	gattggcgct	atttatcctg	gaaatagtga	tactaactac	180
aaccagaagt	tcaagggcaa	ggccaaactg	actgcagtca	catctgccag	cactgcctac	240
atggagctca	gcagcctgac	aatgaggac	gctgcggtct	atcactgtac	aagatcgggg	300
gacctaactg	gggggcttgc	ttactggggc	caagggactc	tggtcactgt	ctctgca	357

<210> 20

<211> 372

<212> DNA

<213> Mus musculus

<400> 20

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tcttgcaagg	cttctggata	caccttcaact	agctactgga	tgcattgggt	gaagcagagg	120
cctggacaag	gccttgagtg	gatcggagag	attgatcctt	ctgatagtta	tacttactac	180
aatcaaaagt	tcaggggcaa	ggccacattg	actgtagaca	aatcctccaa	cacagcctac	240
atgcaactca	gcagcctgac	atctgaggac	tctgcggtct	attactgttc	aagatcaaat	300
ctgggtgatg	gtcactaccg	gtttcctgcg	tttcccttact	ggggccaagg	gactctggtc	360
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<210> 21

<211> 372

<212> DNA

<213> Mus musculus

<400> 21

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cctggacaag	gccttgaatg	gattggtaca	attgaccctt	ctgatagtga	aactcactac	180
aatctacagt	tcaaggacac	ggccacattg	actgtagaca	aatcctccag	cacagcctac	240
atgcagctca	gcagcctgac	atctgaggac	tctgcggtct	attattgtat	aagaggcgcc	300

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tctctatagtt cctatagtta ctgggcctgg ttgcttact ggggcccaagg gactctggtc 360
actgtctctg ca 372
<210> 22
<211> 463
<212> PRT
<213> Mus musculus
<400> 22
Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
1 5 10 15
Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30
Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45
Ser Arg Tyr Ala Met Ser Trp Val Arg Gln Ile Pro Glu Lys Ile Leu
50 55 60
Glu Trp Val Ala Ala Ile Asp Ser Ser Gly Gly Asp Thr Tyr Tyr Leu
65 70 75 80
Asp Thr Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn
85 90 95
Thr Leu His Leu Gln Met Arg Ser Leu Arg Ser Glu Asp Thr Ala Leu
100 105 110
Tyr Tyr Cys Val Arg Gln Gly Gly Ala Tyr Trp Gly Gln Gly Thr Leu
115 120 125
Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
130 135 140
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
145 150 155 160
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
165 170 175
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
180 185 190
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
195 200 205
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
210 215 220
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
225 230 235 240
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
245 250 255
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
260 265 270
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
275 280 285
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
290 295 300
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305 310 315 320
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
325 330 335
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
340 345 350
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
355 360 365
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu

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370	375	380
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala	Val Glu Trp Glu Ser Asn	
385	390	395
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr	Pro Pro Val Leu Asp Ser	400
	405	410
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg		415
	420	425
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu		430
	435	440
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		445
	450	455
		460

<210> 23

<211> 114

<212> PRT

<213> Mus musculus

<400> 23

Glu Val His Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly	
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Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr	10
	20
Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val	25
	30
Ala Ala Ile Asn Asn Asn Gly Asp Asp Thr Tyr Tyr Leu Asp Thr Val	35
	40
Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr	45
	50
Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys	55
	60
Val Arg Gln Gly Gly Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val	65
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	75
	80
	85
	90
	95
	100
	105
	110

Ser Ala

<210> 24

<211> 470

<212> PRT

<213> Mus musculus

<400> 24

Met Gly Trp Asn Trp Ile Phe Ile Leu Ile Leu Ser Val Thr Thr Gly	
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Val His Ser Glu Val Gln Leu Gln Ser Gly Pro Glu Leu Val Lys	10
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Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe	25
	30
Thr Gly Tyr Tyr Met His Trp Val Lys Gln Ser Pro Glu Lys Ser Leu	35
	40
Glu Trp Ile Gly Glu Ile Asn Pro Ser Thr Gly Gly Thr Thr Tyr Asn	45
	50
Gln Lys Phe Lys Ala Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser	55
	60
Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val	65
	70
	75
	80
	85
	90
	95
	100
	105
	110
Tyr Tyr Cys Ala Arg Arg Gly Gly Leu Thr Gly Thr Ser Phe Phe Ala	115
	120
	125
Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys	

	130					135				140					
Gly 145	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly 160
Gly 175	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn
Val 225	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp
Val 305	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly
Val 320	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn
Gln 385	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu
Ser 465	Leu	Ser	Pro	Gly	Lys										
<210>	25														
<211>	118														
<212>	PRT														
<213>	Mus musculus														
<400>	25														
Gln 1	Val	Thr	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Ile	Leu	Gln	Pro	Ser	Gln
Thr	Leu	Ser	Leu	Thr	Cys	Ser	Phe	Ser	Gly	Phe	Ser	Leu	Ser	Thr	Tyr
Gly	Met	Gly	Val	Gly	Trp	Ile	Arg	Gln	Pro	Ser	Gly	Met	Gly	Leu	Glu

Trp	Leu	Ala	Asn	Ile	Trp	Trp	Tyr	Asp	Ala	Lys	Tyr	Tyr	Asn	Ser	Asp
50					55					60					
Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Thr	Ser	Asn	Asn	Gln	Val
65					70				75					80	
Phe	Leu	Lys	Ile	Ser	Ser	Val	Asp	Thr	Ser	Asp	Thr	Ala	Thr	Tyr	Tyr
			85						90					95	
Cys	Ala	Gln	Met	Gly	Leu	Ala	Trp	Phe	Ala	Tyr	Trp	Gly	Gln	Gly	Thr
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Leu	Val	Thr	Val	Ser	Ala										
		115													

<210> 26  
 <211> 118  
 <212> PRT  
 <213> Mus musculus  
 <400> 26

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			20					25					30		
Gly	Met	Gly	Val	Gly	Trp	Ile	Arg	Gln	Pro	Ser	Gly	Lys	Gly	Leu	Glu
		35					40					45			
Trp	Leu	Ala	Asn	Ile	Trp	Trp	Asn	Asp	Asp	Lys	Tyr	Tyr	Asn	Ser	Ala
50					55					60					
Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Thr	Ser	Asn	Asn	Gln	Val
65					70				75					80	
Phe	Leu	Lys	Ile	Ser	Ser	Val	Asp	Thr	Ala	Asp	Thr	Ala	Thr	Tyr	Tyr
			85						90					95	
Cys	Ala	Gln	Ile	Gly	Tyr	Phe	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr
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Thr	Leu	Thr	Val	Ser	Ser										
		115													

<210> 27  
 <211> 471  
 <212> PRT  
 <213> Mus musculus  
 <400> 27

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			20					25					30		
Pro	Gly	Gly	Thr	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Ser	Thr	Phe
		35					40					45			
Ser	Asn	Tyr	Ala	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu
50					55					60					
Glu	Trp	Val	Ala	Ala	Ile	Asp	Ser	Asn	Gly	Gly	Thr	Thr	Tyr	Tyr	Pro
65					70				75					80	
Asp	Thr	Met	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn
			85						90					95	
Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Phe
			100					105					110		
Tyr	His	Cys	Thr	Arg	His	Asn	Gly	Gly	Tyr	Glu	Asn	Tyr	Gly	Trp	Phe
		115					120					125			
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Ala	Ser	Thr
	130					135					140				



Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser
145					150					155					160
Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu
				165					170						175
Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His
			180					185					190		
Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser
		195					200					205			
Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys
	210					215					220				
Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu
225					230					235					240
Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro
				245					250					255	
Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys
			260					265					270		
Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val
		275					280					285			
Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp
	290					295					300				
Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr
305					310					315					320
Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp
				325					330					335	
Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu
			340					345					350		
Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg
		355					360					365			
Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys
	370					375					380				
Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp
385					390					395					400
Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys
				405					410					415	
Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser
			420					425					430		
Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser
		435					440					445			
Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser
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465					470										

<210> 28

<211> 122

<212> PRT

<213> Mus musculus

<400> 28

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			20					25					30		
Ala	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val
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Ala	Ala	Ile	Asn	Ser	Asn	Gly	Gly	Thr	Thr	Tyr	Tyr	Pro	Asp	Thr	Met

	50					55					60				
Lys 65	Asp	Arg	Phe	Thr	Ile 70	Ser	Arg	Asp	Asn	Ala 75	Lys	Asn	Thr	Leu	Tyr 80
Leu	Gln	Met	Ser	Ser 85	Leu	Arg	Ser	Glu	Asp 90	Ser	Ala	Leu	Tyr	Tyr 95	Cys
Thr	Arg	His	Asn 100	Gly	Gly	Tyr	Glu	Asn 105	Tyr	Gly	Trp	Phe	Ala 110	Tyr	Trp
Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala						
			115					120							
<210>	29														
<211>	470														
<212>	PRT														
<213>	Mus musculus														
<400>	29														
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Val	Tyr	Ser	Glu 20	Val	Gln	Leu	Gln	Gln 25	Ser	Gly	Thr	Val	Leu 30	Ala	Arg
Pro	Gly	Ala	Ser 35	Val	Lys	Met	Ser 40	Cys	Lys	Ala	Ser	Gly 45	Tyr	Thr	Phe
Thr	Gly 50	Tyr	Trp	Met	Arg	Trp 55	Val	Lys	Gln	Arg	Pro 60	Gly	Gln	Gly	Leu
Glu 65	Trp	Ile	Gly	Ala	Ile 70	Tyr	Pro	Gly	Asn	Ser 75	Asp	Thr	Thr	Tyr 80	Asn
Gln	Lys	Phe	Lys 85	Gly	Lys	Ala	Lys	Leu 90	Thr	Ala	Val	Thr	Ser 95	Val	Ser
Thr	Ala	Tyr	Met 100	Glu	Leu	Ser	Ser 105	Leu	Thr	Asn	Glu	Asp	Ser 110	Ala	Val
Tyr	Tyr	Cys 115	Ser	Arg	Ser	Gly 120	Asp	Leu	Thr	Gly	Gly 125	Phe	Ala	Tyr	Trp
Gly	Gln 130	Gly	Thr	Leu	Val 135	Thr	Val	Ser	Thr	Ala	Lys 140	Ala	Ser	Thr	Lys
Gly 145	Pro	Ser	Val	Phe 150	Pro	Leu	Ala	Pro	Ser	Ser 155	Lys	Ser	Thr	Ser	Gly
Gly	Thr	Ala	Ala 165	Leu	Gly	Cys	Leu	Val 170	Lys	Asp	Tyr	Phe	Pro 175	Glu	Pro
Val	Thr	Val 180	Ser	Trp	Asn	Ser	Gly 185	Ala	Leu	Thr	Ser	Gly 190	Val	His	Thr
Phe	Pro	Ala 195	Val	Leu	Gln	Ser	Ser 200	Gly	Leu	Tyr	Ser 205	Leu	Ser	Ser	Val
Val	Thr 210	Val	Pro	Ser	Ser 215	Ser	Leu	Gly	Thr	Gln 220	Thr	Tyr	Ile	Cys	Asn
Val 225	Asn	His	Lys	Pro 230	Ser	Asn	Thr	Lys	Val	Asp 235	Lys	Lys	Val	Glu	Pro
Lys	Ser	Cys	Asp 245	Lys	Thr	His	Thr	Cys	Pro 250	Pro	Cys	Pro	Ala	Pro	Glu
Leu	Leu	Gly 260	Gly	Pro	Ser	Val	Phe 265	Leu	Phe	Pro	Pro	Lys	Pro 270	Lys	Asp
Thr	Leu 275	Met	Ile	Ser	Arg	Thr 280	Pro	Glu	Val	Thr	Cys 285	Val	Val	Val	Asp
Val	Ser 290	His	Glu	Asp 295	Pro	Glu	Val	Lys	Phe	Asn 300	Trp	Tyr	Val	Asp	Gly
Val 305	Glu	Val	His	Asn 310	Ala	Lys	Thr	Lys	Pro	Arg 315	Glu	Glu	Gln	Tyr	Asn

Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	
				325					330					335		
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	
			340					345					350			
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	
		355					360					365				
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	
	370					375					380					
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	
385					390					395					400	
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	
			405					410						415		
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	
			420				425						430			
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	
		435				440						445				
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	
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465					470											

<210> 30

<211> 119

<212> PRT

<213> Mus musculus

<400> 30

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Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Gly	Tyr	
		20					25					30				
Trp	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	
	35					40						45				
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Ser	Asp	Thr	Asn	Tyr	Asn	Gln	Lys	Phe	
50					55					60						
Lys	Gly	Lys	Ala	Lys	Leu	Thr	Ala	Val	Thr	Ser	Ala	Ser	Thr	Ala	Tyr	
65				70				75						80		
Met	Glu	Leu	Ser	Ser	Leu	Thr	Asn	Glu	Asp	Ala	Ala	Val	Tyr	His	Cys	
			85					90					95			
Thr	Arg	Ser	Gly	Asp	Leu	Thr	Gly	Gly	Leu	Ala	Tyr	Trp	Gly	Gln	Gly	
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Thr	Leu	Val	Thr	Val	Ser	Ala										
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<210> 31

<211> 124

<212> PRT

<213> Mus musculus

<400> 31

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Ser	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	
		20					25					30				
Trp	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	
	35					40						45				
Gly	Glu	Ile	Asp	Pro	Ser	Asp	Ser	Tyr	Thr	Tyr	Tyr	Asn	Gln	Lys	Phe	
50					55					60						

Arg Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr  
65 70 75 80  
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
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Ser Arg Ser Asn Leu Gly Asp Gly His Tyr Arg Phe Pro Ala Phe Pro  
100 105 110  
Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala  
115 120

<210> 32  
<211> 124  
<212> PRT  
<213> Mus musculus  
<400> 32

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Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr  
20 25 30  
Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45  
Gly Thr Ile Asp Pro Ser Asp Ser Glu Thr His Tyr Asn Leu Gln Phe  
50 55 60  
Lys Asp Thr Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr  
65 70 75 80  
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
85 90 95  
Ile Arg Gly Ala Phe Tyr Ser Ser Tyr Ser Tyr Trp Ala Trp Phe Ala  
100 105 110  
Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala  
115 120

<210> 33  
<211> 717  
<212> DNA  
<213> Mus musculus  
<400> 33

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<211> 336  
<212> DNA  
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catgagtctc	caaggcttct	catcaagtat	gcttcccagt	ccatctctgg	gatcccttcc	180
aggttcagt	gcaatggatc	agggacagat	ttcactctca	gtatcaacag	tgtggagact	240
gaagattttg	gaatgtattt	ctgtcaacag	agtaacatct	ggtcgctcac	gttcggtgct	300

gggaccaagc tggagctgaa a 321

<210> 43

<211> 333

<212> DNA

<213> Mus musculus

<400> 43

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caacagaaac caggacagcc acccaaactc ctcattctatg gtgcatccaa cgtagaatct 180  
ggggtccttg ccagggtttag tggcagtggg tctgggacag acttcagcct caacatccat 240  
cctgtggagg aggatgatat tgcaatgtat ttctgtcagc aaagtaggaa ggttccgtat 300  
acgttcggat cggggaccaa gctggaaata aaa 333

<210> 44

<211> 238

<212> PRT

<213> Mus musculus

<400> 44

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Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu  
35 40 45  
Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro  
50 55 60  
Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser  
65 70 75 80  
Gly Ala Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr  
85 90 95  
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys  
100 105 110  
Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
115 120 125  
Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
130 135 140  
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
145 150 155 160  
Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
165 170 175  
Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
180 185 190  
Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
195 200 205  
Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
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<211> 112

<212> PRT

<213> Mus musculus

<400> 45

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Asp	Gly	Lys	Thr	Tyr	Leu	Asn	Trp	Leu	Leu	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			
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65					70					75					80
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Ile	Tyr	Tyr	Cys	Trp	Gln	Gly
				85					90					95	
Thr	His	Phe	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys
			100					105					110		

<210> 46

<211> 238

<212> PRT

<213> Mus musculus

<400> 46

Met	Ser	Pro	Val	Gln	Phe	Leu	Phe	Leu	Leu	Met	Leu	Trp	Ile	Gln	Glu
1				5					10					15	
Thr	Asn	Gly	Asp	Val	Val	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Ser	Val
			20					25					30		
Thr	Ile	Gly	Gln	Pro	Ala	Ser	Ile	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu
		35					40					45			
Leu	Tyr	Ser	Asn	Gly	Lys	Thr	Tyr	Leu	Asn	Trp	Leu	Gln	Gln	Arg	Pro
	50				55					60					
Gly	Gln	Ala	Pro	Lys	His	Leu	Met	Tyr	Gln	Val	Ser	Lys	Leu	Asp	Pro
65					70				75						80
Gly	Ile	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Glu	Thr	Asp	Phe	Thr
				85					90					95	
Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Tyr	Cys
			100					105					110		
Leu	Gln	Ser	Thr	Tyr	Tyr	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu
		115				120						125			
Glu	Leu	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro
	130					135					140				
Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu
145					150					155					160
Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn
				165					170					175	
Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser
			180					185					190		
Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala
		195					200					205			
Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly
	210					215					220				
Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys		
225						230				235					

<210> 47

<211> 108

<212> PRT

<213> Mus musculus

<400> 47

Asp	Ile	Lys	Met	Thr	Gln	Ser	Pro	Ser	Ser	Met	Tyr	Ala	Ser	Leu	Gly
1				5					10					15	



Glu	Arg	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asp	Ile	Asn	Asn	Tyr
			20					25					30		
Leu	Ser	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Lys	Ser	Pro	Lys	Thr	Leu	Ile
		35					40					45			
Tyr	Arg	Ala	Asn	Arg	Leu	Val	Asp	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				
Ser	Gly	Ser	Gly	Gln	Asp	Tyr	Ser	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Tyr
65					70					75				80	
Glu	Asp	Met	Gly	Ile	Asn	Tyr	Cys	Leu	Gln	Cys	Asp	Glu	Phe	Pro	Pro
				85					90					95	
Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys				
			100					105							

<210> 48  
 <211> 112  
 <212> PRT  
 <213> Mus musculus  
 <400> 48

Asp	Val	Val	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu	Gly
1				5					10					15	
Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		
Asn	Gly	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65					70					75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Phe	Cys	Ser	Gln	Ser
				85					90					95	
Thr	His	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		

<210> 49  
 <211> 234  
 <212> PRT  
 <213> Mus musculus  
 <400> 49

Met	Arg	Pro	Ser	Ile	Gln	Phe	Leu	Gly	Leu	Leu	Leu	Phe	Trp	Leu	His
1				5					10					15	
Gly	Val	Gln	Cys	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser
			20					25					30		
Ala	Ser	Leu	Gly	Gly	Lys	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asp
		35					40					45			
Ile	Asn	Lys	Asn	Ile	Val	Trp	Tyr	Gln	His	Lys	Pro	Gly	Lys	Gly	Pro
	50					55					60				
Arg	Leu	Leu	Ile	Trp	Tyr	Thr	Ser	Thr	Leu	Gln	Pro	Gly	Ile	Pro	Ser
65					70					75				80	
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Arg	Asp	Tyr	Ser	Phe	Ser	Ile	Ser
				85					90					95	
Asn	Leu	Glu	Pro	Glu	Asp	Ile	Ala	Thr	Tyr	Tyr	Cys	Leu	Gln	Tyr	Asp
			100					105					110		
Asn	Leu	Pro	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg
		115					120					125			
Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln
		130					135					140			

Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr
145					150				155						160
Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser
				165					170					175	
Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr
			180					185					190		
Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys
		195				200						205			
His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro
	210					215					220				
Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys						
225					230										

<210> 50  
 <211> 107  
 <212> PRT  
 <213> Mus musculus  
 <400> 50

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Leu	Gly
1				5				10						15	
Gly	Lys	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asp	Ile	Asn	Lys	Asn
			20					25					30		
Ile	Ile	Trp	Tyr	Gln	His	Lys	Pro	Gly	Lys	Gly	Pro	Arg	Leu	Leu	Ile
		35					40					45			
Trp	Tyr	Thr	Ser	Thr	Leu	Gln	Pro	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				
Ser	Gly	Ser	Gly	Arg	Asp	Tyr	Ser	Phe	Ser	Ile	Ser	Asn	Leu	Glu	Pro
65					70					75					80
Glu	Asp	Ile	Ala	Thr	Tyr	Tyr	Cys	Leu	Gln	Tyr	Asp	Asn	Leu	Pro	Arg
				85					90					95	
Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys					
			100					105							

<210> 51  
 <211> 239  
 <212> PRT  
 <213> Mus musculus  
 <400> 51

Met	Arg	Phe	Ser	Ala	Gln	Leu	Leu	Gly	Leu	Leu	Val	Leu	Trp	Ile	Pro
1				5				10						15	
Gly	Ser	Thr	Ala	Asp	Ile	Val	Met	Thr	Gln	Ala	Ala	Phe	Ser	Asn	Pro
			20					25					30		
Val	Thr	Leu	Gly	Thr	Ser	Thr	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser
		35					40					45			
Leu	Leu	His	Ser	Asn	Gly	Ile	Thr	Tyr	Leu	Tyr	Trp	Tyr	Leu	Gln	Lys
	50					55					60				
Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Gln	Met	Ser	Asn	Leu	Ala
65					70					75					80
Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Ser	Ser	Gly	Ser	Gly	Thr	Asp	Phe
				85					90					95	
Thr	Leu	Arg	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr
			100					105					110		
Cys	Ala	Gln	Asn	Leu	Glu	Leu	Pro	Tyr	Thr	Phe	Gly	Ser	Gly	Thr	Lys
	115						120					125			
Leu	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro
	130					135					140				

Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu
145					150					155					160
Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp
			165						170					175	
Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp
		180						185					190		
Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys
	195					200					205				
Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln
	210					215				220					
Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	
225					230					235					

<210> 52

<211> 112

<212> PRT

<213> Mus musculus

<400> 52

Asp	Ile	Val	Met	Thr	Gln	Ala	Ala	Phe	Ser	Asn	Pro	Val	Thr	Leu	Gly
1			5					10						15	
Thr	Ser	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser	Leu	Leu	His	Ser
		20					25					30			
Asn	Gly	Ile	Thr	Tyr	Leu	Tyr	Trp	Phe	Leu	Gln	Lys	Pro	Gly	Gln	Ser
	35					40					45				
Pro	Gln	Leu	Leu	Ile	Tyr	Gln	Met	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro
	50					55				60					
Asp	Arg	Phe	Ser	Ser	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Arg	Ile
65				70					75					80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ala	Gln	Asn
			85					90					95		
Leu	Glu	Leu	Pro	Tyr	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100					105						110		

<210> 53

<211> 107

<212> PRT

<213> Mus musculus

<400> 53

Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Val	Thr	Pro	Gly
1			5					10						15	
Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	Ala	Ser	His	Ser	Ile	Ser	Asn	Phe
		20					25					30			
Leu	His	Trp	Tyr	Pro	Gln	Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	Leu	Ile
	35					40					45				
Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly
	50					55				60					
Asn	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Thr
65				70					75					80	
Glu	Asp	Phe	Gly	Met	Tyr	Phe	Cys	Gln	Gln	Ser	Asn	Ile	Trp	Ser	Leu
			85					90					95		
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys					
		100					105								

<210> 54

<211> 111

<212> PRT

<213> Mus musculus

<400> 54  
 Asp Ile Val Leu Thr Gln Ser Pro Thr Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15  
 Gln Ser Val Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu Tyr Tyr  
 20 25 30  
 Gly Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45  
 Lys Leu Leu Ile Tyr Gly Ala Ser Asn Val Glu Ser Gly Val Pro Ala  
 50 55 60  
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His  
 65 70 75 80  
 Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser Arg  
 85 90 95  
 Lys Val Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys  
 100 105 110

<210> 55

<211> 333

<212> DNA

<213> Mus musculus

<400> 55

cagatccagt	tggagcagtc	tggacctgag	ctgaagaagc	ctggagagac	agtcaagatc	60
tcctgcaagg	cttctgggta	tattttcaga	gactattcaa	tgcactgggt	gaagcaggct	120
ccaggaaagg	gtttaaagtg	gatgggctgg	ataaacactg	agacgggtga	gccaacatat	180
gcagatgact	tcaagggacg	gtttgccttc	tctttggaaa	cctctgccag	caactgcctat	240
ttgcagatca	acaacctcaa	aaatgaggac	acggctacat	atttctgtac	tagcctttac	300
tggggccaag	ggactctggt	cactgtctct	gca			333

<210> 56

<211> 372

<212> DNA

<213> Mus musculus

<400> 56

cagggtcactc	tgaaagagtc	tggccctggg	atattgcagc	cctcccagac	cctcagtcctg	60
acttggttctt	tctctggggt	ttcactgagc	acttatggta	tgggtgtagg	ttggattcgt	120
cagccttcag	ggaaggggtct	ggagtggctg	gccaacattt	ggtggcatga	tgataagtac	180
tataactcag	ccctgaagag	ccggctcaca	atctccaagg	atatctccaa	caaccaggta	240
ttcctcaaga	tctccagtgt	ggacactgca	gataactgcca	catactactg	tgctcaaata	300
gccctcgcgt	ataataagta	cgaaggcttt	tttgcctttct	ggggccaagg	gactctggtc	360
actgtctctg	ca					372

<210> 57

<211> 345

<212> DNA

<213> Mus musculus

<400> 57

cagggttcaac	tgcagcagtc	tggggctgag	ctggtgaggc	ctggggcttc	agtgaagctg	60
tcctgcaagg	cttcgggcta	cacatttact	gactatgaaa	tgcactgggt	gaagcagaca	120
cctgtgcatg	gcctaaaatg	gattggagct	cttgatccta	aaactgggtga	tactgcctac	180
agtcagaagt	tcaagggcaa	ggccacactg	actgcagaca	aatcctccag	cacagcctac	240
atggagctcc	gcagcctgac	atctgaggac	tctgccgtct	attactgtac	aagattctac	300
tcctatactt	actggggcca	agggactctg	gtcactgtct	ctgca		345

<210> 58

<211> 357

<212> DNA

<213> Mus musculus

<400> 58

gaggtgcagc	ttgttgagac	tggtggagga	ctggtgcagc	ctgaaggggtc	attgaaactc	60
tcatgtgcag	cttctggatt	cagcttcaat	atcaatgcca	tgaactgggt	ccgccaggct	120
ccaggaaagg	gtttggaatg	ggttgctcgc	ataagaagtg	aaagtaataa	ttatgcaaca	180
tattatggcg	attcagtga	agacagggttc	accatctcca	gagatgattc	acaaaacatg	240
ctctatctac	aaatgaacaa	cttgaaaact	gaggacacag	ccatatatta	ctgtgtgaga	300
gaggtaaacta	catcgtttgc	ttattggggc	caaggggactc	tggtcactgt	ctctgca	357

<210> 59

<211> 369

<212> DNA

<213> Mus musculus

<400> 59

gaggtgcagc	ttgttgagac	tggtggagga	ttggtgcagc	ctaaaggggtc	attgaaactc	60
tcatgtgcag	cctctggatt	caccttcaat	gccagtgcc	tgaactgggt	ccgccaggct	120
ccaggaaagg	gtttggaatg	ggttgctcgc	ataagaagta	aaagtaataa	ttatgcaata	180
tattatgccg	attcagtga	agacagggttc	accatctcca	gagatgattc	acaaagcatg	240
ctctatctgc	aaatgaacaa	cttgaaaact	gaggacacag	ccatgtatta	ctgtgtgaga	300
gatccgggct	actatggtaa	cccctgggtt	gcttactggg	gccaagggac	tctggtcact	360
gtctctgca						369

<210> 60

<211> 111

<212> PRT

<213> Mus musculus

<400> 60

Gln	Ile	Gln	Leu	Glu	Gln	Ser	Gly	Pro	Glu	Leu	Lys	Lys	Pro	Gly	Glu
1				5					10					15	
Thr	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ile	Phe	Arg	Asp	Tyr
			20					25					30		
Ser	Met	His	Trp	Val	Lys	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Lys	Trp	Met
		35					40					45			
Gly	Trp	Ile	Asn	Thr	Glu	Thr	Gly	Glu	Pro	Thr	Tyr	Ala	Asp	Asp	Phe
	50					55					60				
Lys	Gly	Arg	Phe	Ala	Phe	Ser	Leu	Glu	Thr	Ser	Ala	Ser	Thr	Ala	Tyr
65					70				75					80	
Leu	Gln	Ile	Asn	Asn	Leu	Lys	Asn	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys
			85					90						95	
Thr	Ser	Leu	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	
			100					105						110	

<210> 61

<211> 124

<212> PRT

<213> Mus musculus

<400> 61

Gln	Val	Thr	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Ile	Leu	Gln	Pro	Ser	Gln
1				5					10					15	
Thr	Leu	Ser	Leu	Thr	Cys	Ser	Phe	Ser	Gly	Phe	Ser	Leu	Ser	Thr	Tyr
			20					25					30		
Gly	Met	Gly	Val	Gly	Trp	Ile	Arg	Gln	Pro	Ser	Gly	Lys	Gly	Leu	Glu
		35					40					45			
Trp	Leu	Ala	Asn	Ile	Trp	Trp	His	Asp	Asp	Lys	Tyr	Tyr	Asn	Ser	Ala
	50					55					60				
Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Ile	Ser	Asn	Asn	Gln	Val
65					70				75					80	
Phe	Leu	Lys	Ile	Ser	Ser	Val	Asp	Thr	Ala	Asp	Thr	Ala	Thr	Tyr	Tyr
				85					90					95	

Cys Ala Gln Ile Ala Pro Arg Tyr Asn Lys Tyr Glu Gly Phe Phe Ala  
 100 105 110  
 Phe Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala  
 115 120

<210> 62  
 <211> 115  
 <212> PRT  
 <213> Mus musculus  
 <400> 62

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr  
 20 25 30  
 Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Lys Trp Ile  
 35 40 45  
 Gly Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys Phe  
 50 55 60  
 Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95  
 Thr Arg Phe Tyr Ser Tyr Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr  
 100 105 110

Val Ser Ala  
 115  
 <210> 63  
 <211> 119  
 <212> PRT  
 <213> Mus musculus  
 <400> 63

Glu Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Glu Gly  
 1 5 10 15  
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Asn Ile Asn  
 20 25 30  
 Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ala Arg Ile Arg Ser Glu Ser Asn Asn Tyr Ala Thr Tyr Tyr Gly Asp  
 50 55 60  
 Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Asn Met  
 65 70 75 80  
 Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Ile Tyr  
 85 90 95  
 Tyr Cys Val Arg Glu Val Thr Thr Ser Phe Ala Tyr Trp Gly Gln Gly  
 100 105 110

Thr Leu Val Thr Val Ser Ala  
 115  
 <210> 64  
 <211> 123  
 <212> PRT  
 <213> Mus musculus  
 <400> 64

Glu Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Lys Gly  
 1 5 10 15  
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Ala Ser

20							25				30							
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val			
35							40				45							
Ala	Arg	Ile	Arg	Ser	Lys	Ser	Asn	Asn	Tyr	Ala	Ile	Tyr	Tyr	Ala	Asp			
50							55				60							
Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Gln	Ser	Met			
65							70				75							
Leu	Tyr	Leu	Gln	Met	Asn	Asn	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Met	Tyr			
85							90				95							
Tyr	Cys	Val	Arg	Asp	Pro	Gly	Tyr	Tyr	Gly	Asn	Pro	Trp	Phe	Ala	Tyr			
100							105				110							
Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala								
115							120											
<210> 65																		
<211> 336																		
<212> DNA																		
<213> Mus musculus																		
<400> 65																		
gatgtttgtga							tgacccagac		tccactcact		ttgtcgggta		cccttggaca		accagcctcc		60	
atctcttgca							agtcaagtca		gagcctctta		catagtgatg		gaaagacatt		tttgaattgg		120	
ttattacaga							ggccaggcca		gtctccaaag		cgcctaatact		atctgggtgtc		tagactggac		180	
tctggagtc							ctgacagggt		cactggcagt		ggatcaggga		cagatttcac		actgaaaatc		240	
agcagagtgg							aggtcgagga		tttgggagtt		tattattgct		gccaaaggta		acattttcct		300	
cggacgttcg							gtggaggcac		caggctggaa		atcaaa							336
<210> 66																		
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<212> DNA																		
<213> Mus musculus																		
<400> 66																		
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tacctgcaga							aaccaggcca		gtctccaaag		ctcctgatct		acaaagtttc		caaccgattt		180	
tctgggggtcc							cagacagggt		cagtggcagt		ggatcaggga		cagatttcac		actcaagatc		240	
agcagagtgg							aggtcgagga		tctgggagtt		tattactgct		ttcaagggtc		acatgtttccg		300	
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<210> 67																		
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<400> 67																		
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tacctgcaga							agccaggcca		gtctccaaag		ctcctgatct		acaaagtttc		caaccgattt		180	
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agcagagtgg							aggtcgagga		tctgggagtt		tatttctgct		ctcaaaatac		acatgtttcct		300	
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<210> 68																		
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atctcctgca							agtctagtaa		gagtctcctg		catagtaatg		gcaacactta		cttgaattgg		120	
ttcctgcaga							ggccaggcca		gtctcctcaa		ctcctgattt		attggatgtc		caaccttgcc		180	

tcaggagtcc	cagacaggtt	cagtggcagt	gggtcaggaa	ctgctttcac	actgagaatc	240
agtagagtgg	aggctgagga	tgtgggtggt	tattactgta	tgcaacatat	agaataccct	300
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<210> 69

<211> 336

<212> DNA

<213> Mus musculus

<400> 69

gatattgtga	tgacgcaggc	tgcattctcc	aatccagtca	ctcttggaac	atcagcttcc	60
atctcctgca	ggctctagtaa	gagtctccta	catagttatg	acatcactta	tttgtattgg	120
tatctgcaga	agccaggcca	gtctcctcag	ctcctgattt	atcagatgtc	caaccttgcc	180
tcaggagtcc	cagacaggtt	cagtagcagt	gggtcaggaa	ctgatttcac	actgagaatc	240
agcagagtgg	aggctgagga	tgtgggtggt	tattactgtg	ctcaaaatct	agaacttcct	300
ccgacgttcg	gtggaggcac	caagctggaa	atcaaa			336

<210> 70

<211> 318

<212> DNA

<213> Mus musculus

<400> 70

caaattgttc	tcacccagtc	tccagcaatc	atgtctgcat	ttccagggga	gaaggtcacc	60
atgacctgca	gtgccagctc	aagtgttagt	tacatgtact	ggtaccagca	gaagtcagga	120
tcctcccca	gactcctgat	ttatgacaca	tccaacctgg	cttctggagt	ccctgttcgc	180
ttcagtggca	gtgggtctgg	gacctcttac	tctctcacia	tcagccgaat	ggaggctgaa	240
gatgctgcca	cttattactg	ccagcagtgg	agtagttacc	cgctcacgtt	cggtggtggg	300
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<210> 71

<211> 112

<212> PRT

<213> Mus musculus

<400> 71

Asp	Val	Val	Met	Thr	Gln	Thr	Pro	Leu	Thr	Leu	Ser	Val	Thr	Leu	Gly
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Gln	Pro	Ala	Ser	Ile	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser
		20					25					30			
Asp	Gly	Lys	Thr	Phe	Leu	Asn	Trp	Leu	Leu	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Lys	Arg	Leu	Ile	Tyr	Leu	Val	Ser	Arg	Leu	Asp	Ser	Gly	Val	Pro
		50				55					60				
Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70					75					80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Tyr	Cys	Cys	Gln	Gly
			85					90						95	
Thr	His	Phe	Pro	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Arg	Leu	Glu	Ile	Lys
			100					105					110		

<210> 72

<211> 112

<212> PRT

<213> Mus musculus

<400> 72

Asp	Val	Leu	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu	Gly
1			5					10						15	
Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Ile	Val	His	Ser
		20					25					30			
Asn	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser



		35					40				45				
Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70						75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly
				85					90					95	
Ser	His	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		

<210> 73  
 <211> 112  
 <212> PRT  
 <213> Mus musculus  
 <400> 73

Asp	Val	Val	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu	Gly
1				5					10					15	
Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
		20						25					30		
Asn	Gly	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70						75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Phe	Cys	Ser	Gln	Asn
				85					90					95	
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		

<210> 74  
 <211> 112  
 <212> PRT  
 <213> Mus musculus  
 <400> 74

Asp	Ile	Val	Met	Thr	Gln	Ser	Ala	Pro	Ser	Val	Pro	Val	Thr	Pro	Gly
1				5					10					15	
Glu	Ser	Val	Ser	Ile	Ser	Cys	Lys	Ser	Ser	Lys	Ser	Leu	Leu	His	Ser
			20					25					30		
Asn	Gly	Asn	Thr	Tyr	Leu	Asn	Trp	Phe	Leu	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Gln	Leu	Leu	Ile	Tyr	Trp	Met	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ala	Phe	Thr	Leu	Arg	Ile
65				70						75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Met	Gln	His
				85					90					95	
Ile	Glu	Tyr	Pro	Phe	Thr	Phe	Gly	Thr	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		

<210> 75  
 <211> 112  
 <212> PRT  
 <213> Mus musculus  
 <400> 75

Asp	Ile	Val	Met	Thr	Gln	Ala	Ala	Phe	Ser	Asn	Pro	Val	Thr	Leu	Gly
1				5					10					15	

Thr	Ser	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser	Leu	Leu	His	Ser
			20					25					30		
Tyr	Asp	Ile	Thr	Tyr	Leu	Tyr	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Gln	Leu	Leu	Ile	Tyr	Gln	Met	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro
		50				55					60				
Asp	Arg	Phe	Ser	Ser	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Arg	Ile
65					70					75					80
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ala	Gln	Asn
				85					90					95	
Leu	Glu	Leu	Pro	Pro	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105						110	

<210> 76

<211> 106

<212> PRT

<213> Mus musculus

<400> 76

Gln	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Phe	Pro	Gly
1				5					10					15	
Glu	Lys	Val	Thr	Met	Thr	Cys	Ser	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met
			20					25					30		
Tyr	Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Ser	Ser	Pro	Arg	Leu	Leu	Ile	Tyr
		35					40					45			
Asp	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Val	Arg	Phe	Ser	Gly	Ser
		50				55					60				
Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Arg	Met	Glu	Ala	Glu
65					70					75					80
Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Tyr	Pro	Leu	Thr
				85					90					95	
Phe	Gly	Gly	Gly	Thr	Glu	Leu	Glu	Leu	Lys						
			100					105							

<210> 77

<211> 345

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse-human chimeric antibody H chain

<400> 77

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tcttgcaagg	cttctggata	caccttcacc	gactatgaaa	tgcaactgggt	gcgacaggcc	120
cctggacaag	ggcttgagtg	gatgggagct	cttgatccta	aaactgggtga	tactgcctac	180
agtcagaagt	tcaagggcag	agtcacgatt	accgcggacg	aatccacgag	cacagcctac	240
atggagctga	gcagcctgag	atctgaggac	acggccgtgt	attactgtgc	gagattctac	300
tcctatactt	actggggcca	gggaaccctg	gtcaccgtct	cctca		345

<210> 78

<211> 345

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse-human chimeric antibody H chain

<400> 78

caggtgcagc	tggtggagtc	tggagctgag	gtgaagaagc	ctggggcctc	agtgaaggtc	60
tcttgcaagg	cttctggata	caccttcacc	gactatgaaa	tgcaactgggt	gcgacaggcc	120
cctggacaag	ggcttgagtg	gatgggagct	cttgatccta	aaactgggtga	tactgcctac	180

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agtcagaagt tcaagggcag agtcacgctg accgcggacg aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtac aagattctac      300
tcctatactt actggggcca gggaaccctg gtcaccgtct cctca                        345
<210> 79
<211> 345
<212> DNA
<213> Artificial Sequence
<220>
<223> Mouse-human chimeric antibody H chain
<400> 79
caggtgcagc tgggtggagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg cttctggata caccttcacc gactatgaaa tgcactgggt gcgacaggcc      120
cctggacaag ggcttgagtg gatgggagct cttgataccta aaactgggtga tactgcctac      180
agtcagaagt tcaagggcag agtcacgctg accgcggaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtac aagattctac      300
tcctatactt actggggcca gggaaccctg gtcaccgtct cctca                        345
<210> 80
<211> 345
<212> DNA
<213> Artificial Sequence
<220>
<223> Mouse-human chimeric antibody H chain
<400> 80
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tcctgcaagg cttctggata caccttcacc gactatgaaa tgcactgggt gcgacaggcc      120
cctggacaag ggcttgagtg gatgggagct cttgataccta aaactgggtga tactgcctac      180
agtcagaagt tcaagggcag agtcacgctg accgcggaca aatccacgag cacagcctac      240
atggagctga gcagcctgac atctgaggac acggccgtgt attactgtac aagattctac      300
tcctatactt actggggcca gggaaccctg gtcaccgtct cctca                        345
<210> 81
<211> 345
<212> DNA
<213> Artificial Sequence
<220>
<223> Mouse-human chimeric antibody H chain
<400> 81
caggtgcagc tgggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg cttctggata caccttcacc gactatgaaa tgcactgggt gcgacaggcc      120
cctggacaag ggcttgagtg gatgggagct cttgataccta aaactgggtga tactgcctac      180
agtcagaagt tcaagggcag agtcacgctg accgcggacg aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtac aagattctac      300
tcctatactt actggggcca gggaaccctg gtcaccgtct cctca                        345
<210> 82
<211> 345
<212> DNA
<213> Artificial Sequence
<220>
<223> Mouse-human chimeric antibody H chain
<400> 82
caggtgcagc tgggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg cttctggata caccttcacc gactatgaaa tgcactgggt gcgacaggcc      120
cctggacaag ggcttgagtg gatgggagct cttgataccta aaactgggtga tactgcctac      180
agtcagaagt tcaagggcag agtcacgctg accgcggaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtac aagattctac      300

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tcctatactt actggggcca gggaaccctg gtcaccgtct cctca
<210> 83
<211> 345
<212> DNA
<213> Artificial Sequence
<220>
<223> Mouse-human chimeric antibody H chain
<400> 83
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tcctgcaagg cttctggata caccttcacc gactatgaaa tgcactgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggagct cttgataccta aaactgggtga tactgcctac 180
agtcagaagt tcaagggcag agtcacgctg accgcggaca aatccacgag cacagcctac 240
atggagctga gcagcctgac atctgaggac acggccgtgt attactgtac aagattctac 300
tcctatactt actggggcca gggaaccctg gtcaccgtct cctca 345
<210> 84
<211> 115
<212> PRT
<213> Artificial Sequence
<220>
<223> Mouse-human chimeric antibody H chain
<400> 84
Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Glu Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Phe Tyr Ser Tyr Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110
Val Ser Ser
115
<210> 85
<211> 115
<212> PRT
<213> Artificial Sequence
<220>
<223> Mouse-human chimeric antibody H chain
<400> 85
Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Glu Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys Phe
50 55 60
Lys Gly Arg Val Thr Leu Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

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Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	
Thr	Arg	Phe	Tyr	Ser	Tyr	Thr	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr
			100					105					110		

Val Ser Ser  
115

<210> 86

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse-human chimeric antibody H chain

<400> 86

Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala
1				5					10					15	
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asp	Tyr
			20					25						30	

Glu	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
		35					40						45		

Gly	Ala	Leu	Asp	Pro	Lys	Thr	Gly	Asp	Thr	Ala	Tyr	Ser	Gln	Lys	Phe
	50					55					60				

Lys	Gly	Arg	Val	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75					80

Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Thr	Arg	Phe	Tyr	Ser	Tyr	Thr	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr
			100					105					110		

Val Ser Ser  
115

<210> 87

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse-human chimeric antibody H chain

<400> 87

Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala
1				5					10					15	
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asp	Tyr
			20					25						30	

Glu	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
		35					40						45		

Gly	Ala	Leu	Asp	Pro	Lys	Thr	Gly	Asp	Thr	Ala	Tyr	Ser	Gln	Lys	Phe
	50					55					60				

Lys	Gly	Arg	Val	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75					80

Met	Glu	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Thr	Arg	Phe	Tyr	Ser	Tyr	Thr	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr
			100					105					110		

Val Ser Ser  
115

<210> 88

<211> 115

<212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> Mouse-human chimeric antibody H chain  
 <400> 88  
 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr  
 20 25 30  
 Glu Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45  
 Gly Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys Phe  
 50 55 60  
 Lys Gly Arg Val Thr Leu Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Thr Arg Phe Tyr Ser Tyr Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr  
 100 105 110

Val Ser Ser  
115

<210> 89  
 <211> 115  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> Mouse-human chimeric antibody H chain  
 <400> 89

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr  
 20 25 30  
 Glu Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45  
 Gly Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys Phe  
 50 55 60  
 Lys Gly Arg Val Thr Leu Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Thr Arg Phe Tyr Ser Tyr Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr  
 100 105 110

Val Ser Ser  
115

<210> 90  
 <211> 115  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> Mouse-human chimeric antibody H chain  
 <400> 90

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr



<210> 94  
 <211> 8  
 <212> PRT  
 <213> homo sapiens  
 <400> 94  
 Gly Asn Ser Gln Gln Ala Thr Pro  
 1 5  
 <210> 95  
 <211> 8  
 <212> PRT  
 <213> homo sapiens  
 <400> 95  
 Gln Gln Ala Thr Pro Lys Asp Asn  
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 <210> 96  
 <211> 8  
 <212> PRT  
 <213> homo sapiens  
 <400> 96  
 Thr Pro Lys Asp Asn Glu Ile Ser  
 1 5  
 <210> 97  
 <211> 10  
 <212> PRT  
 <213> homo sapiens  
 <400> 97  
 Ala Thr Pro Lys Asp Asn Glu Ile Ser Thr  
 1 5 10  
 <210> 98  
 <211> 10  
 <212> PRT  
 <213> homo sapiens  
 <400> 98  
 Pro Lys Asp Asn Glu Ile Ser Thr Phe His  
 1 5 10  
 <210> 99  
 <211> 10  
 <212> PRT  
 <213> homo sapiens  
 <400> 99  
 Asp Asn Glu Ile Ser Thr Phe His Asn Leu  
 1 5 10  
 <210> 100  
 <211> 10  
 <212> PRT  
 <213> homo sapiens  
 <400> 100  
 Glu Ile Ser Thr Phe His Asn Leu Gly Asn  
 1 5 10  
 <210> 101  
 <211> 27  
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Gly Asn Ser Gln Gln Ala Thr Pro Lys Asp Asn Glu Ile Ser Thr Phe  
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 His Asn Leu Gly Asn Val His Ser Pro Leu Lys  
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Ser Thr Phe His Asn Leu Gly Asn Val His Ser Pro Leu Lys  
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Asn Tyr Ala Met Ser  
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Ala Ile Asn Asn Asn Gly Asp Asp Thr Tyr Tyr Leu Asp Thr Val Lys  
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Asp

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Gln Gly Gly Ala Tyr  
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Thr Tyr Gly Met Gly Val Gly  
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Met Gly Leu Ala Trp Phe Ala Tyr

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Gly Tyr Trp Met His
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Ser Tyr Ala Met Ser
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Ser Tyr Trp Met His  
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 Gly

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 Phe Tyr Ser Tyr Thr Tyr  
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 Ile Asn Ala Met Asn  
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 Val Lys Asp

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 Glu Val Thr Thr Ser Phe Ala Tyr  
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 Ala Ser Ala Met Asn  
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Leu Tyr  
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 Arg Ala Asn Arg Leu Val Asp  
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 Leu Gln Cys Asp Glu Phe Pro Pro Trp Thr  
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 Arg Ala Ser His Ser Ile Ser Asn Phe Leu His

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Tyr Ala Ser Gln Ser Ile Ser
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Gln Gln Ser Asn Ile Trp Ser Leu Thr
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Arg Ala Ser Glu Ser Val Glu Tyr Tyr Gly Thr Ser Leu Met Gln
1           5           10           15
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Gly Ala Ser Asn Val Glu Ser
1           5
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Gln Gln Ser Arg Lys Val Pro Tyr Thr
1           5
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Ser Gln Asn Thr His Val Pro Pro Thr
1           5
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Lys Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Asn
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 Arg Ser Ser Lys Ser Leu Leu His Ser Tyr Asp Ile Thr Tyr Leu Tyr  
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 Ser Ala Ser Ser Ser Val Ser Tyr Met Tyr  
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Leu Val Ser Arg Leu Asp Ser
1          5
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Cys Gln Gly Thr His Phe
1          5
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Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
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Phe Gln Gly Ser His Val Pro Trp Thr
1          5
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<223> PCR primer
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cttgtagaca gtgacggaaa cacctat
<210> 173
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<212> DNA
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<223> PCR primer
<400> 173
ataggtgttt ccgtcactgt gtacaag
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<212> PRT
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<223> mutant antibody L chain
<400> 174
Arg Ser Ser Gln Ser Leu Val His Ser Asn Ala Asn Thr Tyr Leu His
1          5          10          15
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<400> 176
Arg Ser Ser Gln Ser Leu Val His Ser Asn Glu Asn Thr Tyr Leu His
1          5          10          15
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1          5          10          15
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Arg Ser Ser Gln Ser Leu Val His Ser Asn His Asn Thr Tyr Leu His
1          5          10          15
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1          5          10          15
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Arg Ser Ser Gln Ser Leu Val His Ser Asn Thr Asn Thr Tyr Leu His
1          5          10          15
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<212> PRT

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<213> Artificial Sequence
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1          5          10          15
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Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Ile Asn Thr Tyr Leu
1          5          10          15
His
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<400> 183
Arg Ser Ser Gln Ser Leu Val His Ser Asn Lys Asn Thr Tyr Leu His
1          5          10          15
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Arg Ser Ser Gln Ser Leu Val His Ser Asn Leu Asn Thr Tyr Leu His
1          5          10          15
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Arg Ser Ser Gln Ser Leu Val His Ser Asn Ser Asn Thr Tyr Leu His
1          5          10          15
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Arg Ser Ser Gln Ser Leu Val His Ser Asn Trp Asn Thr Tyr Leu His
1          5          10          15
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<211> 16
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Arg Ser Ser Gln Ser Leu Val His Ser Asn Tyr Asn Thr Tyr Leu His
1          5          10          15
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<400> 188
Arg Ser Ser Gln Ser Leu Val His Ser Asn Arg Asn Thr Tyr Leu His
1          5          10          15
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<400> 189
Arg Ser Ser Gln Ser Leu Val His Ser Asn Val Asn Thr Tyr Leu His
1          5          10          15
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<223> mutant antibody L chain
<400> 190
Arg Ser Ser Gln Ser Leu Val His Ser Asn Pro Asn Thr Tyr Leu His
1          5          10          15
<210> 191
<211> 112
<212> PRT
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<223> mutant antibody L chain
<400> 191
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1          5          10          15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20          25          30
Asn Ala Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35          40          45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Asn
85          90          95

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Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
100 105 110

<210> 192

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant antibody L chain

<400> 192

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly  
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
20 25 30

Asn Asp Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Asn  
85 90 95

Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
100 105 110

<210> 193

<211> 112

<212> PRT

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<223> mutant antibody L chain

<400> 193

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly  
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
20 25 30

Asn Glu Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Asn  
85 90 95

Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
100 105 110

<210> 194

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant antibody L chain

<400> 194

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly  
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser

			20					25					30			
Asn	Phe	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser	
		35					40					45				
Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro	
	50					55					60					
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile	
65				70						75					80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn	
				85					90					95		
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	
			100					105						110		

<210> 195

<211> 112

<212> PRT

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<220>

<223> mutant antibody L chain

<400> 195

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly	
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Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser	
			20					25				30				
Asn	His	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser	
		35					40					45				
Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro	
	50					55					60					
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile	
65				70						75					80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn	
				85					90					95		
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	
			100					105						110		

<210> 196

<211> 112

<212> PRT

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<220>

<223> mutant antibody L chain

<400> 196

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly	
1				5					10					15		
Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser	
			20					25				30				
Asn	Asn	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser	
		35					40					45				
Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro	
	50					55					60					
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile	
65				70						75					80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn	
				85					90					95		
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	
			100					105						110		

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 Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
 20 25 30  
 Asn Thr Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
 35 40 45  
 Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
 50 55 60  
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
 65 70 75 80  
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Asn  
 85 90 95  
 Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
 100 105 110

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 Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
 20 25 30  
 Asn Gln Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
 35 40 45  
 Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
 50 55 60  
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
 65 70 75 80  
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Asn  
 85 90 95  
 Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
 100 105 110

<210> 199  
 <211> 112  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> mutant antibody L chain  
 <400> 199  
 Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly  
 1 5 10 15  
 Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
 20 25 30  
 Asn Ile Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
 35 40 45



Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70						75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn
			85					90						95	
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105						110	

<210> 200

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant antibody L chain

<400> 200

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5					10					15	
Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		
Asn	Lys	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35				40						45			
Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70						75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn
			85					90						95	
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105						110	

<210> 201

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant antibody L chain

<400> 201

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5					10					15	
Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		
Asn	Leu	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35				40						45			
Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70						75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn
			85					90						95	
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105						110	

<210> 202

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant antibody L chain

<400> 202

```
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1          5          10          15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
          20          25          30
Asn Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
          35          40          45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
          50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Asn
          85          90          95
Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
          100          105          110
```

<210> 203

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant antibody L chain

<400> 203

```
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1          5          10          15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
          20          25          30
Asn Trp Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
          35          40          45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
          50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Asn
          85          90          95
Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
          100          105          110
```

<210> 204

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant antibody L chain

<400> 204

```
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1          5          10          15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
          20          25          30
Asn Tyr Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
          35          40          45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
          50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
```

65					70					75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn
				85					90					95	
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		

<210> 205  
 <211> 112  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> mutant antibody L chain  
 <400> 205

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5					10					15	
Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		
Asn	Arg	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65					70				75					80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn
				85				90						95	
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		

<210> 206  
 <211> 112  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> mutant antibody L chain  
 <400> 206

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5					10					15	
Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		
Asn	Val	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65					70				75					80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn
				85				90						95	
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		

<210> 207  
 <211> 112  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> mutant antibody L chain  
 <400> 207

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1				5					10					15	
Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Ser
			20					25					30		
Asn	Pro	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70						75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ser	Gln	Asn
				85					90					95	
Thr	His	Val	Pro	Pro	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		